

Nuclear power stations for sale?

The newly re-elected British government says that it plans to denationalize its electricity utilities, but that it has not yet decided how. Here, gratuitously, is some advice.

THE re-election last Thursday of Mrs Margaret Thatcher's British government is not, in itself, a surprise, although the size of its majority seems to have startled even those who will enjoy it for the next five years. On the face of things, the development will further disappoint those in Britain who have been wringing their hands over the condition of the British research enterprise for the past several years, *Nature* among them. Yet that could be too gloomy a view. The simple arithmetical argument that the government will be almost as able as in the previous parliament to subdue opposition with its voting strength is only half the truth. The other is that its quite remarkable electoral achievement may have two psychological consequences — that of making it at once more daring, or more radical, and that of giving it a confidence belied by its defensive impulsiveness of the past few years which, among other things, may allow it to listen to its critics. Since there is no prospect of a change in the political scene for close on five years (if then), that at least is how the critics might best arrive at an accommodation with events.

The obvious agenda on which the new government appears ready to embark has education significantly near the top of the list, and for practical reasons. The plan that there should be a nationally uniform curriculum in secondary schools requires that the government should have power to tell local education authorities, hitherto paramount in this respect, what their schools should teach. Plans centrally to support the polytechnics require legislation allowing them to be removed from the control of the same authorities. And if the University Grants Committee is to be replaced by a University Funding Council, with or without power to make contracts with universities, there will have to be legislation to that effect. Whether the new government will persist with its old ambitions to abolish academic tenure remains to be seen, but there is no doubt that the parliamentary row about the education bill will centre on the proposals, sprung on a startled British electorate towards the end of the election, that any school might opt out of local education authority control if its governors or its students' parents so wished. Behind the dust-storm these proposals are certain to raise, there should be ample opportunity for safeguarding against some of the proposals on higher education to which the previous government nervously attached itself.

The starting-point for reasoned argument should be an understanding of why the British government appears to be moved by discontent with the research enterprise for which it pays the bill (see *Nature* 327, 448; 1987). It believes that universities are not properly accountable for the subsidies they enjoy and that, in their pursuit of research, they have been waywardly neglectful of the needs of industry. (Separately, the British government also inclines to the view that its research councils are managed indecisively.) But the parliamentary argument will be fought over the detailed proposals which, in the government's opinion, have been devised to correct these evils. With a majority of more than a hundred in the House of Commons, there is no reason why the government should give way on the details of its policy unless it is persuaded that its objectives might be better attained in some other way.

What other courses of action might there be? Anomalously,

universities would be more acutely aware of their financial responsibility for spending public money wisely if they were more, and not less, free to determine their own affairs. The weakness in present arrangements is that universities know that there are strong political reasons why they will not be allowed to go out of business, even if they should grossly mismanage their affairs. The proposed system of financing by means of contracts with the funding council, while adding an element of arbitrariness to university planning, will further dilute academics' responsibility for their institutions' survival. Why not push the system in the opposite direction by means of arrangements to ensure that their survival as institutions is in their own hands? One simple way of doing this would be to increase the money value of students' tuition fees, at present only nominal and paid by the central government direct to universities through the intermediary of local education authorities. A government whose radicalism centres around the belief that the market works wonders must surely be open to persuasion in that direction, but would universities take the risk? □

Is health affordable?

All governments are worried about rising health costs; are health economists a sufficient answer?

FOR most of the past 40 years, many US physicians and some US politicians have described the British National Health Service as "socialized medicine", partly because the services of a large proportion of British physicians are now formally contracted by the state. So what is to be made of this decade's tendency in the United States for physicians to be salaried employees of health-care corporations whose revenues are derived from the now bewildering variety of health plans to which individuals belong, to which their employers often contribute and which are also subsidized by the federal government either directly or through tax exemptions (reckoned to cost more than \$50,000 million this year)? And what is to be said of the tendency of the US Congress to limit the fees the government's own health care schemes, Medicare and Medicaid, will pay physicians and hospitals for particular services? Is that creeping socialized medicine, or otherwise a sign of how some future British government may shed the administrative burden of running Britain's hospitals and other health-care services?

The changing machinery of health care in the market economy of the United States is succinctly described by Alain Enthoven, a consultant to Health and Social Services Secretary Joseph Califano during the Carter administration, in a symposium published this week (*Health economics — prospects for the future*, G. Teeling Smith (ed.); Croom Helm for the Office of Health Economics). The overt agenda is the undoubted importance of economics in understanding the benefits of health care and in controlling the costs thereof, but the book is also a valuable summary of how different countries handle the costs of health care. The arresting statistic is that spending on health care in the United States increased from 5.3 per cent of Gross National Product in 1960 to 10.7 per cent in 1983, and continues



to increase. Enthoven, evidently an optimist, believes that the pace of growth will now be slower, as government regulation and market forces limit some of the component costs — physicians' salaries and/or the substitution of paramedics for physicians, for example. But he acknowledges that the United States has not solved the problem of the indigent (many of the 35 million people without medical insurance) and that the future is clouded because there is no knowing whether the technology of applied biology (called medicine) will keep people healthy for longer or instead create more expensive ways of staying alive.

That is the uncertainty the British National Health Service was meant to resolve, and which seems to have been overlooked in the run-up to last week's British general election. Mr Aneurin Bevan, the Labour politician who created the health service in 1947, made it a pillar of his case that no-charge access for all would reduce even the then meagre need to spend on medicine; he was right in guessing that infections would quickly disappear from among the causes of death, but he reckoned without coronary by-pass operations and the like. Enthoven might have strengthened his case by guessing that some of these may also disappear when, for example, biotechnologists have found the best ways of delivering clotting enzymes to blood-clots. Yet there are likely always to be as many expensive life-preserving innovations as radically cheaper versions of existing treatments. Enthoven's economic optimism is no more compelling than Bevan's more qualitative opinion about the long-term economic benefits of preventive medicine.

It was inevitable that the conundrum should have featured in the British general election, but its appearance was curiously muffled. The Labour opposition party (as it remains) complained repeatedly about the shortage of resources in the National Health Service. The government's standard reply was 'at resources have not been cut, but rather increased. Both disputants are correct. Although, at one stage, the prime minister, Mrs Margaret Thatcher, was reminding radio listeners that her government has increased the annual budget of the National Health Service from £9,000 million in 1979 to £21,000 million now, inflation has eaten away at the figures. In real terms, spending has increased by 26 per cent over the past 14 years, while much of the extra has been necessary to maintain recruitment of nurses and other paramedical professionals. Meanwhile, with private spending on health care merely 5 per cent of the total, Britain is spending both a smaller proportion of national income (just under 7 per cent) and a smaller absolute sum each year (an average of £400 per head of population) on health care than are countries such as the United States, West Germany and those of Scandinavia.

In such circumstances, it is inevitable that those who can afford it will purchase medical care privately, usually through insurance schemes. Thatcher ran into trouble from her opponents during the election by allowing that she does just that. The counter-complaint, that access to medical services should be determined exclusively by need, and not by a person's ability to pay, is tenable only when the resources available are at least enough to meet all needs — which in Britain at present would probably mean an extra £10,000 million. Nobody, during the election, was talking that big.

So does that mean that the principles of the British National Health Service must be abandoned? Even those who voted last week for Thatcher have made plain their attachment to the National Health Service, whatever its inadequacies, and for good reason. Over 40 years, "socialized medicine" has contributed powerfully to the civility of British life by ensuring that children, the elderly and the chronically sick are well catered for, and that the poor do not need to pay. It is high time that countries such as the United States more energetically followed suit. But the device by which people in jobs purchase health care separately through insurance schemes, far from being subversive of the principles of the health services, is a means by which extra resources can in present political circumstances be found.

The logic of Thatcher's personal position requires that she and her government should do more to encourage the development of private medical insurance in Britain. (Payments are not tax-exempt in Britain, but were specifically excluded from the list of tax deductions snatched away by last year's US Tax Reform Act.) The end result might not be very different from that in the United States and West Germany, with the reservation that those in need continue to receive the treatment they need.

The health economists contributing to this week's symposium would approve. In strictly economic terms, the need in Britain and similar places is merely to recruit extra resources for the health services; whether they come from taxation or from private contributions to insurance schemes is irrelevant to a first approximation (but, to a second approximation, may bear on the efficiency with which health services are managed and on the more contentious question, in Britain, of whether public and private hospitals should be physically separate). But, in the long run, there will be no escape from the dilemma that the cost of innovations that prolong life will always increase so as to exceed what the richest countries can afford; the marginal cost of increasing people's expectation of life must be an increasing function which, at some point, may even be infinite.

Is one resolution of this difficulty to be found in the contribution to this symposium from Professor Alan Williams, of the University of York, who argues for allowing patients threatened with death to make intelligent choices between alternative treatments on the basis of objective studies of the effect of their outcome on the quality and additional extent of life that people may then expect? That would be a great simplification of present dilemmas. If people's rational choices of alternative treatments had the effect of containing the marginal cost of longevity, the administrators would be happy. Such a development would also be consonant with the modern rediscovery of the inevitability of death. The snags are that many physicians would regard such considerations as an extra and even intolerable responsibility which would, more generally, raise ethical questions such as those arising in the consideration of euthanasia. Williams is nevertheless right to ask that the questions should be faced, preferably on the basis of data yet to be gathered about the quality of life of people surviving traumatic medical treatments. The trouble, even if the data were available and used, is that the marginal cost of treatment for all would still be greater than societies believe they can afford. What, people must one day ask, is to happen then? □

Buying nuclear power

The new British government should think hard about its plan to denationalize electricity.

MR Cecil Parkinson, energy minister in the new British government (see p. 543), is right to say that there is some thinking to do before the nationalized British electricity utilities can be sold. That is to put it mildly. Over more than half a century, Britain's pride has been its investment in a national grid, a network of high-voltage transmission lines for distributing electric power over a small patch on the surface of the Earth whose economic benefit has been that consumers could gather electrons economically from power stations sited more or less anywhere in the country. Integration has been the name of the game so far, but selling off an integrated network and its attendant power stations to a private monopoly is an invitation to abuse. Logically, the new private company should own the grid and should buy the electricity it distributes (on appropriately long-term contracts) from whoever is willing to take the risk of building power stations (some of which will be nuclear). That way, consumers would be protected, while plant manufacturers would have an incentive to compete with each other. Otherwise, it is the managers of the present system who will benefit. Which way will a market-oriented government decide? □

British election brings no great changes yet

- Nationalized electricity industry for sale
- Possibility of academic tenure row ahead

London

THE chief problems facing British science have not been much changed by last week's general election, when the Conservative government was returned with a majority of 102 over all other parties combined — a majority much greater than predicted by most of the pre-election opinion polls.

The prime minister, Mrs Margaret Thatcher, has made only limited changes in the composition of her cabinet. Mr Kenneth Baker, the Secretary of State for Education and Science appointed just a year ago, remains in his post.

One impending change at the Department of Education and Science has not, however, been decreed by Thatcher but forced from within. At the weekend, Mr George Walden, minister of state at the department with responsibility for higher education, said that he planned to resign from the government for "personal reasons".

The most striking change in the make-up of the new British government, that of the appointment of Mr Cecil Parkinson as Secretary of State for Energy, will however affect the British technical community. Parkinson, who resigned from his post as Secretary of State for Trade and Industry after the 1983 election, in the wake of a scandal involving his personal secretary, will among other things be responsible for selling the nationalized British electricity utilities to private owners. He said at the weekend that legislation to give effect to these intentions will not be ready during the first year of the new Parliament.

Parkinson's previous and potentially influential job at the Department of Trade and Industry will be filled, in the new government, by Lord Young, who, as Secretary of State for Employment during the past three years, has been responsible for the way in which training and retraining schemes outside the formal educational system have flourished.

His new department's potential influence stems from the diversity of the industries which it sponsors and the potentially useful size of its research and development budget (nearly £400 million a year). But Young, who has a business background, could also be influential in making real the government's ambition to see industry giving more support to basic research, possibly through tax incentive schemes.

More immediately, Young and his deputy, Mr Kenneth Clark, who is also being moved from the Department of Employment, will have to decide and defend Britain's policy towards the European Community's research programme (still on ice) and in space research and development. The British National Space Centre, set up last year, has been waiting since September for a response to its 15-year national space plan.

Mr Nicholas Ridley, Secretary of State for the Environment in the previous government, retains that role, but will probably be mostly occupied with plans to change the basis of local government taxation. Mr Michael Jopling, Secretary of State for Agriculture in the previous government and responsible in that role for decisions about the amounts of radioactivity that might be contained in sheepmeat offered for sale as well as for the hasty announcement of a policy to provide farmers with an incentive to care for the environment, not just to produce more food, will not be a member of the new government.

The new British government will first show its hand in relation to science in the Queen's speech next week, marking the opening of the new parliament. Baker's education bill is certain to be controversial. Taking Britain's 26 polytechnics out of local authority control seems relatively uncontroversial; whether the new government will also move to make academic tenure unconstitutional remains to be seen.

Administratively, the new government's major changes are likely to be embodied in response to the report of the House of Lords Select Committee on Science and Technology, promised before the summer parliamentary recess in mid-July. Most probably there will be changes of the function and constitution of the two chief advisory committees on research and development, the Advisory Board for the Research Councils (ABRC), which divides the civil science budget between its recipient research councils, and the Advisory Council on Applied Research and Development (ACARD), intended to have a broader role which has not hitherto been fulfilled. For many, the crucial question will be whether the government accepts the recommendation that some minister should take political responsibility for the administration of science.

Simon Hadlington

Lightning strikes NASA twice

Washington

A freak accident at the Wallops Island (Virginia) Flight Facility has left the US National Aeronautic and Space Administration (NASA) wondering whether it must contend not only with congressional displeasure and a loss of public approval but also with malignant acts of nature. During a brief but intense thunderstorm on the evening of 9 June, a lightning strike launched three rockets, apparently by generating an electric pulse in firing cables running to the control room.

Two 4-ft-long test rockets set off on their prescribed course, plunging into the Atlantic Ocean several miles away, but the third, a 16-ft Orion rocket, was lying on the ground at the time and skipped a hundred yards into the sea.

This mishap occurs only a few months after an Atlas-Centaur rocket launched from Cape Canaveral was struck by lightning and had to be destroyed (see *Nature* 326, 428; 1987). Some newspapers poked particular fun at NASA because the lost rocket was carrying a payload to investigate electrical activity during a thunderstorm.

But Leslie Hale of Pennsylvania State University, the principal investigator on the experiment, emphasizes that because the instruments on board were to measure electric field and conductivity in the vicinity of lightning, the launch inevitably entailed a certain risk. And he points out a greater irony: information gained from flights such as these can help to make rocket-launching in general safer, yet public scrutiny of NASA is now so intense that even low-key programmes such as the launches from Wallops Island are in danger of being held up.

Several dozen small rockets such as the Orion are launched from Wallops every year, and last week's failure was the first in 1,300 flights. The rockets, military surplus items as much as 30 years old, cost essentially nothing, says Hale, and provide a cheap way of putting many small experiments aloft. Indeed, Hale and his many colleagues regard NASA's small-rocket programme as a safe, cost-effective and enormously productive scientific activity, and are worried that the continuing concentration on spectacular projects with large budgets will damage their research.

Meanwhile, the brief embarrassment over the Wallops Island incident will pass, and Hale looks forward to the day when the media no longer telephone after every launch to see if anything went wrong. But he did note one further irony; his rocket was struck by lightning one day before the anniversary of Ben Franklin's famous kite-flying experiment.

David Lindley

Rumours of Japan's research on superconductors worries US

Washington

RUMOURS that Japan has launched lavish programmes of high-temperature superconductor research helped heighten the feeling of anxiety at the first US congressional hearing on the new materials, held last week. Representative Don Ritter (Republican, Pennsylvania) reported that Japanese government and industry may be spending "\$4 million a week" on superconductor research, against the \$30 million that Erich Bloch, director of the National Science Foundation, estimates that the US federal government will spend in the whole of this financial year.

The evidence of a Japanese representative, Shinroku Saito, president of the Technological University of Nagaoka, that Japan's Ministry of International Trade and Industry (MITI) had not actually launched a national programme since the new superconductors were discovered last November did little to ease the worries of witnesses testifying before the House Committee on Science, Space and Technology. Some still called for a US national programme to counter Japan's (even though it seems not to exist).

H. Kent Brown, an engineering professor at Massachusetts Institute of Technology, fresh from a trip to Japan, confirmed that there is no national programme. But little comfort was to be had. He explained that Japanese corporations are so confident of winning the race to turn the new superconductors into commercial products that they could not be bothered to ask for MITI's help.

While the Japanese government may not be as deeply involved with industry as some US politicians imagine (see *Nature* 327, 356; 1987), the "vertical integration of Japanese industry, low cost capital and tremendous skill in processing of bulk ceramics" to which Ritter drew attention certainly must give US industry cause for concern. Witnesses at the hearing groped for a combination of basic and applied research that would break Japan's run of successes in automobiles, electronic goods and semiconductors.

All agreed that the United States still leads the world in basic research, but that transferring technology from laboratories to industry is the problem. William Graham, science adviser to the president, assured researchers that the president was behind them and offered a massive federal conference on the commercial applications of superconductivity (which will directly follow a conference with the same theme at Argonne National Laboratory in July). But congressmen questioning Graham were not satisfied that his good intentions would lead to actions.

A more radical suggestion, that one national laboratory be dedicated to the development of superconductors, came from Senator Pete Domenici (Republican, New Mexico). He is canvassing schemes for the formation of new consortia between a national laboratory and industry that would encourage technology transfer. Ritter agreed and called for a "coordinated 'Team America' strategy" bringing together industry, government and universities, and removing barriers to cooperation within industry by changes in anti-trust and patent laws.

Domenici has already introduced a bill to create a National Commission on Commercial and National Defense Applications of Superconductors. But IBM's Praveen Chaudhari argued last week that

government should help the whole technology development process by "appropriately supportive policies" rather than target specific technologies, and that it should limit direct investment to basic research and infrastructure. The "fruitful commercialization of technology", he said, is "the role and responsibility of private industry".

Despite the lavish praise they received, those carrying out basic research clearly had woes of their own. Paul Chu, whose discovery of superconductivity above 90 K fuelled the present excitement, worried about the health of the scientific community and stressed that important innovations could only come with support that left researchers "free to explore the unthinkable". But perhaps worrying about the international race that superconductor research has become, he added that "if breakthroughs could occur every day, our feeble hearts could probably not handle the stress". **Alun Anderson**

Moscow Radio raises questions over missing Soviet scientist

London

THE case of Vladimir Aleksandrov, the Soviet computer specialist who disappeared from a peace conference in Madrid at the end of March 1985, is in the headlines again. In a broadcast on Moscow Radio's English-language service, his wife Alestina claimed that she still believes her husband to be alive somewhere, but that during his last trip to the United States before his disappearance he had encountered "difficulties" from the US security services. Furthermore, she said, the Spanish authorities had issued his visa for the peace conference only when it was half over.

Aleksandrov, who had worked on the Soviet model of 'nuclear winter', had visited the United States on a number of occasions. According to his wife's account, on his last trip a stamp had been placed in his passport indicating that he was not allowed to work with the computer which he and his US colleagues had previously used for nine months. He had to give a written promise that he would not speak at public conferences nor have any contact with the media, she said. Moreover, a security agent was "invariably present at his desk", his movements around the United States were monitored, and when he stayed for a night with a friend, he was informed by "alert security agents" that he had no right to leave his hotel room at night. All this, Mrs Aleksandrova said, forced his colleagues at the Lawrence Livermore Laboratory to apologize for the conditions in which he had to work.

The Moscow Radio announcer suggested that Mrs Aleksandrova's story

about her husband's last US trip might "contain some hints at least as to where Aleksandrov should be looked for". But a former colleague, Georgii Stanchikov, was unwilling to accept this suggestion. Although the nuclear winter theory has opponents "and not only among scientists", he said, the theory was well known and no one would think that the removal of Aleksandrov would stop the debate. "I cannot name a person or organization that could be interested in the disappearance of Aleksandrov", Stanchikov concluded.

Colleagues of Aleksandrov from the Lawrence Livermore Laboratories confirm that, during his last visit there in January 1985, security procedures were somewhat stricter than hitherto. But this was due to a general tightening-up of US restrictions and was not directed against Aleksandrov personally. They have no knowledge, however, of any official US prohibitions on his making public statements, and suggest that if any such restriction was imposed on his contacts with the media, it came not from the US authorities but from SCOPE, the environmental group which had sent him there to compare US and Soviet models for the nuclear winter, and which would not have wanted any premature leaking of this work. In fact, Aleksandrov gave at least two lectures during this visit, one at Oregon State University, and one in Los Angeles. Moreover, as on previous occasions, he stayed at the home of one of his US colleagues. Livermore scientists suggest that Mrs Aleksandrova's comments may be the result of some kind of misunderstanding. **Vera Rich**

ESA presents united front to competitors at Paris air show

Paris

THE 37th international exhibition of aeronautics, the Salon du Bourget, is in full swing at Le Bourget, the site of the former airport outside Paris. It is a time for tub-thumping as exhibitors from 31 countries, with more than 200 exhibits, try to persuade buyers that their products are the best. Within the arena, member states of the European Economic Community (EEC) wear their two hats — European and national — somewhat precariously.

With the Ariane series of rocket launchers, and the planned Ariane 5—Hermes shuttle, the European Space Agency (ESA) presents a united front to competitors, particularly the United States, but national pride nevertheless shines through. Determined to have a returnable, manned shuttle in operation by the end of the century, ESA is going ahead with development of the French-inspired Hermes, to be launched by an Ariane 5 rocket, also still on the drawing board.

A full-scale model of Hermes is on display, showing radical design modifications in the light of the Challenger disaster last year. To comply with ESA demands that Hermes should have an ejectable cockpit, in the event of problems with the launcher, its manufacturers, Aérospatiale, have reduced the cockpit from 17 m³ to 5 m³. The number of crew that can be carried has, as a result, been reduced from six to two, with space for a scientific specialist as 'passenger'. The slimmed-down Hermes could be launched from an Ariane 5 with a 21-tonne capacity, whereas the previous version required an unwieldy 25-tonne capacity launcher.

Details of Europe's space programme for the next few years will be discussed at a meeting of ESA partners on 22–23 June and it is expected that some member states will be asked to increase their budget contribution by up to 100 per cent. Apart from Ariane 5 and Hermes, ESA intends to go ahead with construction of its Columbus laboratory module, to be attached to the NASA space station, as well as its Pallas free-flying man-tended laboratory. In an interview with the national daily *Le Monde*, M. Frédéric d'Allest, president of the French Centre Nationale d'Etudes Spatiales (CNES), expressed the hope that ESA would favour an integrated approach to funding these projects, as Ariane, Hermes and Columbus may not, sensibly, be considered as autonomous entities. To keep pace with a maiden Ariane 5 launch in 1995, a Hermes launch in 1997 and the installation of the first Columbus element in 1996, the current £1,200 million annual budget will need to increase to £1,700

million by 1995.

Meanwhile, the Salon du Bourget fuels dreams of what air travel will be like for passengers in the next century. For the French, hopes are that the Avion Grande Vitesse (AGV) will pip its rivals to the post as the hypersonic passenger carrier of the future. Aérospatiale AGV will use statoreactor engine technology to take passengers from a conventional runway to an altitude of 30 km, with speeds up to Mach 5, bringing New York a mere hour

away from Paris. AGV's rivals are the US 'Orient Express' and the German Sanger, capable of launching a payload into orbit as well as carrying passengers. The British HOTOL (horizontal take-off and landing).

With its revolutionary, but untested, air-breathing Rolls-Royce RB 546 engine, until recently in competition with Hermes as the vehicle to link up with Columbus, also belongs to this future generation and serves as a reminder that no single European manufacturer is ever likely to be able to finance its pet project without the support of its EEC partners and this may mean a choice has to be made.

Peter Coles

India confident about its indigenous missile programme

New Delhi

INDIA, which successfully tested indigenously made surface-to-air missiles (SAM) recently, is confident that its missile programme will go ahead despite the seven-nation export ban on critical components and technologies that came into effect on 16 April. The Indian Minister of State for Defence, Mr Arun Singh, told parliament that his scientists were engaged in analysis of the new export controls announced by the United States, Britain, Japan, Italy, West Germany, Canada and France. He said that "with the emphasis given, and the success achieved in indigenization over the years, it is expected that these new controls will not make any significant impact on our programme."

Five different missiles are under development at the Defence Research Development Laboratory (DRDL) in Hyderabad, one of the 40 laboratories under the Defence Research Development Organisation (DRDO).

Trishul, the first Indian-made truck-

mounted SAM, can spring into action in eight seconds and destroy an aircraft 9 km away. DRDO has conducted four flights of Trishul and hopes to put it into production by 1990. The ground control equipment that can handle more than one target at a time will be simultaneously produced by the state-owned Bharat Electronics Limited under licence from a company in the Netherlands.

A long-range (25 km) version of Trishul called Akash, developed at DRDL, is to be test-flown next year. Akash can be fired from truck, aircraft and also from a ship against sea-skimming missiles.

In the next few weeks, DRDL proposes to test its first surface-to-surface missile called Prithvi. It is reported to have a range of 250 km and to be capable of delivering a one-tonne warhead. It is also the first Indian-made missile with an inertial guidance system. Prithvi is liquid-fuelled; Akash and Trishul use solid propellants with very high specific impulse.

The defence scientists have also developed what they call the third-generation fire-and-forget anti-tank missile, called Nag. It is supposed to knock out a tank 4 km away. The fifth missile under development is called Agni (which means fire). Details of this missile are classified.

According to the Defence Ministry, all these missiles will have entered the production phase by 1993–94 when the US\$1,500 million missile range at Balia-pal on the east coast is expected to be ready.

The Indian missile programme has grown independently of its space programme and DRDO has its own test range, workshops and facilities for producing propellants and electronic systems. It is claimed that DRDO is self-sufficient in most of the critical systems. DRDO will soon start making inertial guidance systems under licence from a French company.

K.S. Jayaraman

Watchmaker rewarded

London

THE unusual choice for this year's Royal Society of Literature's Heinemann Award is Richard Dawkins's *The Blind Watchmaker*. A science book has never previously won the prize. Dawkins's book, a popular exposition of neo-darwinism, follows *The Selfish Gene* (1976) and *The Extended Phenotype* (1981). It was published late last year by Longman in Britain and Norton in the United States and has sold a healthy 40,000–50,000 copies in hardback.

To his royalty payments Dawkins can now add the Heinemann Award's £3,000 "for the encouragement of genuine contributors to literature".

T.L.

Germanies' environmental agreement

Munich

WEST Berliners and West Germans now have grounds to hope for relief from the clouds of coal smoke wafting over from East Germany. With the initialling of an East/West German environmental agreement in Bonn on 10 June, the two governments have agreed to work together on reducing air and water pollution and minimizing damage to forests. The pact will be signed during the still-speculative visit later this year of Erich Honecker, general secretary of East Germany's Communist Party, to West Germany.

The environmental agreement with East Germany is just the latest in a series of arrangements between West Germany and Soviet bloc countries in 1987. It follows agreements on nuclear power, health and environment made with the Soviet Union in March and April. Long-awaited pacts between East and West Germany on scientific cooperation and nuclear power are expected to be signed soon.

One important political hurdle cleared in these latest negotiations is the inclusion of the West German Umweltbundesamt (Federal Environmental Office or UBA), an institute in West Berlin; East Germany has agreed for the first time that researchers at the UBA may participate in collaborative projects, even though the East German government does not accept the location of the institute in West Berlin, still officially administered by the four powers victorious in the Second World War. As in the nuclear agreement with the Soviets, negotiators agreed upon the so-called *ad personam* solution in which conference invitations to West Berlin researchers are addressed to them privately.

Researchers from the two states will meet regularly over the next three years to discuss ways to reduce emissions containing sulphur dioxide and nitrogen oxides. East Germany is particularly dependent on pollution-intensive high-sulphur brown coal and faces costs of millions of West German marks to refit their power plants or use other energy sources.

The onus is now on West Germany to provide hard currency for these conversions, which are already technically possible. Sources in several ministries said that there are no plans for financial help on a grand scale, but West German finance minister Martin Bangemann (Free Democrat) has mentioned more than once the willingness of West German companies to offer to East Germany free pilot projects in power generation and smoke clean-up. These projects are one step closer to realization. Steven Dickman

Animal patent dispute out in the open at congressional hearings

Washington

GROUPS demanding legislation to reverse the US Patent and Trademark Office's controversial decision to permit the patenting of genetically engineered animals had a first chance to air their views at a congressional hearing last week. They responded with vigorous objections to the Patent Office decision, on both ethical and economic grounds.

In its evidence to the subcommittee on courts, civil liberties and the administration of justice, the Patent Office stuck to the position that the decision it took in April (see *Nature* 326, 729; 1987) was not a determination of public policy. All that had been done, Assistant Commissioner for Patents Rene Tegtmeyer argued, was to implement the decision, made seven years earlier by the Supreme Court in the case of *Diamond versus Chakrabarty*, that patent matter was to "include anything under the Sun made by man". That decision did not "leave much room to refuse to consider living things as patentable subject matter if they were a product of human intervention".

An animal welfare group, the Humane Society, was more concerned with broad ethical principles than legal history. Its view is that the patenting of life "violates the basic ethical precepts of civilized society and unleashes the potential for

uncontrollable and unjustified animal suffering".

Perhaps more telling was the Humane Society's argument, amplified by others, that the patenting of animal models would encourage a competitive rather than a collaborative research atmosphere to "the ultimate detriment of the public's best interests". Costly research would be duplicated and scientific progress be inhibited. In the end, according to environmental groups represented at the hearing, that would mean that only big companies would be able to keep going. Farmers, reliant on them for the supply of more productive animals, would be forced to pay higher prices which would be passed on to the consumer. The corporations would get rich quick while everyone else would suffer. And, the League of Rural Voters pointed out, that would transfer power over agriculture from the nation's farmers to giant agribusiness.

Biotechnology industry representatives did not agree. They saw the granting of patents as an enormous stimulus that would revitalize research and add massively to the varieties offered to the farmer. In biotechnology, small companies are still the rule and investors would be more likely to come forward if they knew that new products could be patented.

Alun Anderson

TPA patent battle continues in court

London

THE spotlight remains focused on proceedings in London's High Court this week, as the UK drug company Wellcome continues its legal efforts to see the revocation of Britain's first biotechnology patent, held on the production of tissue plasminogen activator (TPA) by the US group Genentech (see *Nature* 327, 450; 1987).

As the case moved into its second week, Genentech lawyers began cross-examining some of the scientists who had provided testimony on behalf of Wellcome. First on the stand was Joe Sambrook, who was at the Cold Spring Harbor Laboratory when he initiated the work that led, by a complex series of events, to Wellcome's process for making TPA. He was followed by Mary-Jane Gething, who now works with Sambrook at the University of Texas Health Science Center in Dallas, Tim Harris of Celltech, which has an independent interest in TPA, and Tom Maniatis of Harvard University but also a founder of Genetics Institute, to which Sambrook turned for collaboration at one stage.

Wellcome seeks to prove that Genentech's process of cloning TPA DNA was not

sufficiently novel or inventive to establish patentability, but is instead 'obvious'. Wellcome is a rival to Genentech in the production of TPA, a clot-dissolving drug with the potential for enormous sales. Wellcome is also arguing that the patent as issued covers all methods of producing TPA and as such is far too broad.

Genentech is expected to start producing its witnesses and evidence later this week, with the court case scheduled to continue all next week. Genentech's case will be aimed at proving that its biotechnological clone is novel and therefore patentable. Senior scientists at the company went into retreat to read about 400 papers and establish their case.

Before the court are stacks of thick files with reports and papers, all carefully arranged — but the profusion of paper often leads to confusion as a witness is asked to turn, for example, to "Bundle 3, Tab H, second page, fifth paragraph, last sentence". After the first week of the case, the judge remarked ruefully that "there must be more papers on TPA than there are authorities on obviousness".

Kathy Johnston

Call for university expansion in West Germany

Munich

THE influential West German science council, the Wissenschaftsrat, has issued a stirring warning to the federal government (and to others who may be within earshot) that university investment should not be reduced. In its seventeenth annual "framework" plan on university construction, issued on 4 June, the Wissenschaftsrat seems to be especially alarmed at budget reductions planned by the federal finance ministry.

The influence of the Wissenschaftsrat derives from its constitutional status as the means by which the federal government, denied responsibility for research under its constitution, brings together other interested parties in executing an agreed policy. The Wissenschaftsrat includes representatives from federal and *Land* (state) governments as well as from industry and basic research. While its "framework plans" carry no official authority, they always serve as a strong guideline for policymakers.

This year's recommendations emphasize the need to finish the institutes and universities begun during the building boom of the 1970s. The Wissenschaftsrat also says that weak areas, such as clinical

research in medicine, need to be supported more strongly.

The pressure on medical faculties (and the increasing unemployment of physicians) should be eased by further reducing the numbers of freshman medical students by 25 per cent, according to the framework plan. And there is a need, the Wissenschaftsrat says, to invest more money in decentralized computers linked in networks among researchers and institutions.

In sounding the call for a total of DM 9,300 million in university investment between 1988 and 1991, the Wissenschaftsrat pointed out that the universities are still enormously overburdened with students. Yet, the Wissenschaftsrat says, the amount spent on teaching and research remained constant, in real terms, between 1980 and 1985.

The timing of the recommendations is significant. The federal government is trying to find ways to cut its budget to allow for the tax reform planned for the years ahead, and the politicians in Bonn are well aware that the numbers of students at the universities and *ochschulen* are expected to decrease dramatically in the mid-1990s.

With its proposal to support more clinical research, the Wissenschaftsrat is attempting to fill a long-recognized gap in the West German research programme. Five additional research groups a year will be supported with DM 2 million each, so that by the fifth year the annual budget will be DM 25–30 million.

The call to reduce the number of medical students derives from a concern about a loss in quality as well as a (barely hidden) desire to reduce the number of doctors coming on the market. Under the West German constitution, the government is not allowed to manipulate the number of medical students based on the demand for doctors.

But because of excessive numbers of applicants, prospective medical students began to be judged on their school grades in the late 1960s. Further cuts could be made if it were shown that the number of students far exceeds the ability of institutions to teach them properly. Kurt-Jürgen Maass at the Wissenschaftsrat points out that the ration of medical students to beds in the teaching hospitals now amounts to 3:5 and that it is "much higher in some universities". In Maass' opinion, reducing the number of students by a quarter to a third would provide "entirely enough" doctors.

Such a decision would be welcomed by professional organizations; the Health Ministry is currently preparing a study to

see whether the reduction is justified.

An official at the federal Education Ministry indicated that the 1988 federal budget, which will probably be hammered out by mid-July, should not reflect deep cuts in support for education. Achieving the DM 9,300 million recommended by 1991 is another matter. **Steven Dickman**

Sex of new embryos known

London

Two British medical teams have succeeded in identifying the sex and detecting hereditary diseases in newly fertilized embryos, raising moral and ethical questions.

The University of Edinburgh's *in vitro* fertilization (IVF) team has devised a test which uses a commercially available DNA probe to identify the male Y chromosomes in embryos 4–8 days old. Seven human embryos were investigated, and 6 of these were positive for Y-chromosome DNA.

The Edinburgh team says the test could be used to test embryos obtained during *in vitro* fertilization treatment, or normally fertilized embryos collected by uterine flushing, and could lead to prenatal diagnosis of sex-linked genetic disorders.

Further research is necessary to perfect techniques to remove a single cell from the embryos, while using cryopreservation before implanting unaffected embryos into the mother's womb.

Gene probes are also being used to detect hereditary diseases in embryos by Professor Robert Winston and his team at London's Hammersmith Hospital IVF unit. Winston says he will be ready to apply the animal-tested technique to humans in "a matter of months", once he obtains approval from the local Ethics of Medical Research Committee and the Voluntary Licensing Authority.

A service to detect genetic disorders and hereditary diseases such as cystic fibrosis, haemophilia, muscular dystrophy and Down's syndrome is expected to be offered by a new £250,000 IVF clinic due to open at Hammersmith Hospital in October.

These successes of the two medical teams have led to reports in British newspapers that parents will soon be able to choose the sex of their test-tube babies, and speculation that it would be possible to change the sex composition of the population. But both teams deny that this is the intention. "Changing the population is not on", says Winston. A member of the Edinburgh team, Dr John West, says, "It certainly wouldn't be ethical to use the method to choose the sex of a baby. But", he admits, "we couldn't prevent the technique being used that way."

Kathy Johnston

New AIDS drug

London

BRITISH scientists who have discovered a new drug for the treatment of AIDS (acquired immune deficiency syndrome) and cancer are seeking financial support from a new company, Medirace, which is soon to be listed on the Stock Exchange.

The drug, called Contracon, is based on changes in the ratio of fatty acids in cell membranes associated with virus infections. Researchers say it seems to act by preventing cells from dividing, and by stopping the penetration or exit of the virus. The work has been met with scepticism by some other researchers, and so far there have been no clinical trials of the effect of the drug on AIDS.

Dr Kosta Apostolov, one of the team based at the Royal Postgraduate Medical School at Hammersmith Hospital, says initial research was funded "from our own pockets", but he says Australian and American backers have now promised about \$1 million for research and development work and clinical trials.

In answer to critics who say the team is jumping on the AIDS bandwagon he says, "it is just coincidence that we found these results at this time". Apostolov says the results of the research will be submitted to a medical journal shortly. Kathy Johnston

DNA fingerprinting at a price at ICI's UK laboratory

London

WITH the opening this month of ICI's UK laboratory for DNA fingerprinting on a commercial basis, the company is moving ahead to start a similar US facility in the near future and is planning other European testing centres.

Operating in Abingdon, Oxfordshire, the UK laboratory is run by Cellmark Diagnostics, a subsidiary of ICI Diagnostics. With a staff of 22, the laboratory can report a result in 3 weeks after the delivery of 5-ml blood samples. About one week is needed to complete the test itself but Cellmark Diagnostics staff will be trying to streamline the test to increase its sensitivity and computerize some of the process.

Japan circumvents whaling ban

London

JAPANESE scientists have told the scientific committee of the International Whaling Commission (IWC) now meeting in Bournemouth, England, that Japan intends to continue harvesting nearly 900 whales a year under a scientific permit.

The IWC has imposed a moratorium on commercial whaling until at least 1990, but under Article VIII of the international convention for the regulation of whaling, a member government of the IWC "may grant to any of its nationals a special permit authorizing that national to kill, take and treat whales for purposes of scientific research".

Japan's announcement that it will kill 825 minke whales and 50 sperm whales a year in the Antarctic has been criticized as a convenient way of circumventing the present ban on commercial whaling. The IWC's scientific committee questions the scientific merit of the Japanese programme.

Iceland and Korea are also carrying out whale-harvesting in the name of scientific research. Although the IWC's scientific committee concluded that Iceland's research programme "would provide only a minimal improvement in our current knowledge with respect to providing management advice", the committee cannot prevent any special scientific permits being issued.

Norway is continuing its commercial whaling programme, in defiance of the moratorium, and conservationists say that under the scientific research loophole, several ex-whaling nations including Brazil and the Philippines are considering carrying out some research of their own.

Kathy Johnston

The laboratory's initial business will come from paternity testing. DNA fingerprinting to a child and its supposed parents is becoming recognized as the best way to determine paternity. The test is likely to settle many cases where disputed relationships are the basis of an immigration dispute.

Cellmark Diagnostics will charge £105 for each test, and can currently perform 250 a week. The test distinguishes individuals on the basis of the permutations in a series of highly variable DNA sequences scattered throughout the chromosomes. The variations are detected after fragmenting the DNA, separating the fragments by size on a gel and detecting them with radioactive DNA probes. Dr Alec Jeffries of the University of Leicester invented the test and the Lister Institute, of which he is a research fellow, holds the patents. ICI has exclusive rights to use the test commercially.

In addition to testing family relationships, DNA fingerprinting has considerable forensic potential. The Home Office Forensic Science Service has already shown that the test can be applied to sperm samples and may therefore be of use in rape cases. It will also be useful in cases where an assailant has left behind blood or tissue. The Leicestershire police are already using the test on blood samples from several thousand males, among whom they hope to identify a murderer they are hunting. There has been a long-running dispute between the Home Office and ICI as to the handling of forensic applications of DNA fingerprinting; "discussions are continuing", says ICI.

Beyond fingerprinting, ICI Diagnostics has interests in the application of DNA tests in diagnosing genetic diseases. DNA probes for several diseases are now available and many more will follow. So far, UK testing has been carried out on a non-commercial basis. Much of the research that has led to the probes and their initial diagnostic applications have been financed by charities, such as the Cystic Fibrosis Research Trust. Three pilot programmes to carry out the tests within the UK National Health Service are nearing completion with no assurance that they will continue or, indeed, be expanded. ICI may offer such tests on a commercial basis. The company also has an eye on methods for testing genetic predispositions and for applying DNA fingerprinting to the identification of cell lines and to establishing animal pedigrees.

Other companies with an interest in DNA-based tests include Lifecodes Corporation, a New York based biotech-

New tide in UK oceanography?

London

THE Institute of Oceanographic Sciences (Bidston), one of Britain's leading oceanographic research centres, was established as an independent laboratory of the Natural Environment Research Council (NERC) on 1 April 1987 and was last week renamed the Proudman Oceanographic Laboratory (POL). This is part of NERC's new-found drive to focus on relevant applied research, but the question is whether this move by NERC will help progress in research to be made.

Dr John Woods, NERC marine sciences director, regards POL as a "key laboratory" for running strategic research programmes. The laboratory is best known for its work on the study of tides and sea-level. Work on deep-sea pressure measurements has provided a greater understanding of tides in the North Atlantic and there is a programme of *in situ* measurements to complement the ERS-1 altimeter data. The work on sea-level measurements is part of the World Ocean Circulation Experiment (WOCE). The three-dimensional modelling of continental shelf circulation performed at Bidston is the basis for the North Sea Project initiated by NERC. This is a strategic research programme to provide the United Kingdom with a prognostic model, with applications to marine pollution and sediment motion.

NERC's support and enthusiasm for research at POL has given rise to optimism about the future, but the laboratory has had a chequered history. It was founded as the Liverpool University Tidal Institute in 1919 by the physical oceanographer Professor Joseph Proudman. In 1969 it was taken over by NERC (and renamed the Institute of Coastal Oceanography and Tides) and, in 1973, linked with the National Institute of Oceanography (Wormley) and the Unit of Coastal Sedimentation (Taunton) to form the Institute of Oceanographic Sciences. (The Taunton laboratory was closed down in 1985.)

According to some academics, NERC's involvement was greeted with optimism in 1969, but was soon followed by disillusion and frustration. Now people will be watching to see whether NERC's latest initiative will fulfil its promise. Philippa Lloyd

nology company with several patents on fingerprinting techniques using different probes, the Boston-based company Collaborative Research, which has been accumulating large numbers of chromosome-specific DNA markers, and Celltech which has "some plans to move into the area of DNA diagnostics but is waiting for the right opportunity", according to chief executive Gerard Fairtlough.

Peter Newmark

British copyright protection law in sight in new parliament

London

THE newly elected Conservative government is expected to devise new copyright and patent legislation early in the next session of parliament, providing protection from piracy for British high-technology industries.

The proposed legislation was outlined in the spring of 1986 with the publication of a white paper (policy document) *Intellectual Property and Innovation* but was jettisoned from the programme of the last parliamentary session for lack of time in the run-up to last week's general election.

Advisers at the Department of Trade and Industry, responsible for framing legislation, were relieved at the respite, because of the complexity of the bill, but continued to work on it as legislation would be necessary irrespective of the political flavour of the new government.

The legislation, according to the government when the white paper was published, "will improve the intellectual property system in the United Kingdom to the benefit of innovative talent and those who enjoy and use the fruits of these talents".

The most recent attempts to study the inadequacies of current legislation began with the committee headed by Mr Justice Whitford, which published its report, *Copyright and Designs Law*, in 1977. The government, which changed from Labour to Conservative in the interim, did not respond until four years later, in the form of a green paper (discussion document) outlining the problems facing the "reform of the law relating to copyright, designs and performers' protection".

By that time, advances in computing, videotape recording and photocopyers since 1977 made many of the government responses to Whitford obsolete. But the industries under threat could not wait for further studies and demanded immediate action. To satisfy the film industry, an amendment to existing legislation was introduced in 1983 making it a crime to handle, sell or process counterfeit video material.

The British computer industry demanded the same immediate protection. Most of the major software and hardware companies formed a lobby group called the Federation Against Software Theft (FAST). The federation, which soon claimed that more than £150 million a year was being lost through illegal copying and commercial piracy, much of it in the Far East, had some success in 1985 with the passing of an amendment to the Copyright Act of 1956, giving protection to computer programs and devising a range of penalties for the offenders. The possession, sale or

hire of pirated software could mean a fine up to £2,000 or imprisonment for up to two months for each offence.

The new legislation package now being drafted will attempt to protect the copyright owners from loss of revenue on a very wide front, hence the complexity of the legislation. It includes the prohibition of home copying of computer programs and video tapes, a 10 per cent levy on audio cassette tapes, more copyright protection for computer programs and the extension of film/broadcasting copyright to television programmes beamed by satellite.

But vested interests within these industries are pressing for even stronger

measures. The music and film industries have been seeking a levy on blank video cassettes and players as compensation for lost revenue; the computer industry wants a simpler and less burdensome method of challenging copyright. And, because of the amount of revenue allegedly lost through illegal copying, FAST will demand that the copyright holder should have the sole right to determine whether a product can be hired or not.

The high-technology industries will also seek protection under international agreements to stifle organized piracy at its source. The Berne Copyright Convention, now 100 years old, provides some copyright protection in more than 75 countries, but many of the developing nations in the Far East are either not signatories or ignore the rules and so remain the primary sources of counterfeit product.

Bill Johnstone

Chinese scientist refused leave to visit United States

London

FANG Lizhi, the astrophysics professor who was expelled from the Chinese Communist Party last January, has been refused permission to visit the United States this year. Declining an invitation for a month-long visit of lectures and joint research at the University of California's Lick Observatory, Fang explained that an official of the Chinese Academy of Sciences had told him that his presence "might raise instabilities among Chinese students studying in the United States".

Fang has, however, been allowed to attend an astrophysics conference in Italy, and is due shortly in Britain for a similar meeting in Cambridge. His situation seems to reflect a fluid situation in the official Chinese attitude to academic dissent. Officially, the Chinese line is, once again, to allow a hundred thoughts to contend, and although, in January, Fang was relieved of his post at the University of Science and Technology in Hefei, there was no official objection when the Chinese Academy of Sciences found him a research post in Beijing. On the other hand, the student disturbances of last December have left the authorities wary of anything that could lead to further unrest in the universities. Furthermore, the Chinese students in the United States have already come under the watchful eye of the home government, by issuing an open letter expressing their concern about events in China. Although, according to He Dongchang, the Vice-Minister of Education, the signatories of the letter had not understood the domestic situation properly, Fang's presence in the United States would clearly constitute too great a risk.

Official Chinese explanations of the December strikes put the blame more and more on Fang: in a recent 'dialogue' with students at the University of Science and Technology (subsequently reported at length in the People's Daily), the deputy secretary of the University's Party Committee stated unequivocally that "it was Fang who encouraged students to strike". Fang himself is saying as little as possible about the events leading up to his expulsion from the party, and, although, during his visit to Italy, he gave an interview for Japanese television, he kept his remarks low-key and tried not to commit himself about his role in the disturbances.

Although students throughout China have endorsed ritual condemnations of Fang's political errors, he still seems to enjoy some sympathy and support in student circles. At the end of last month, students of Beijing University elected Fang's wife, Li Shuxian, an associate professor of physics, as a delegate to the local district People's Congress. These congresses are the only government body elected by popular vote (in this case, three candidates being elected from a slate of four, nominated by the students). Li Shuxian received 8,639 votes out of a total of 9,689, with the second and third most popular candidates receiving 5,473 and 5,264 respectively. There was a 90 per cent turnout, which Chinese sources describe as "unprecedented". In recent years, turnout has twice been so low that the election has had to be repeated a second time (and in one case a third time) to secure a mandate. Officials of Beijing University attribute the new high turnout to the students having "gradually increased their awareness of elections".

Vera Rich

Ensuring scientific integrity

SIR—Recent articles in *Nature*¹⁻⁵ have drawn attention to the problems that have been created by scientific fraud, suggesting that fraud is a disease that could even now be afflicting identifiable groups of scientists.

As with other infections, I should like to know where it originated, the causative agent and methods for its detection, treatment and prevention. Just because the cases reported in *Nature* have all been from the United States does not necessarily mean that fraud is of North American provenance — our colleagues there may simply be better at diagnosing the disease in the world's largest population of scientists. Stewart and Feder⁴ believe that the causative agent is the pressure to publish, but Braunwald⁵ feels that an underlying psychiatric problem could often be responsible.

There appears to be some disagreement on the best methods of detection, but the analysis of one case indicates that a sound knowledge of statistics is helpful². (Ironically, widespread statistical expertise could also render detection more difficult.) Published accounts suggest that detection depends heavily on delation and hindsight for success. It is clear that diagnosis is not straightforward^{4,5}. Automatic ostracism of the culprit from the scientific body seems to be the longstanding therapy.

Serious consideration needs to be given to prophylaxis. First, I suggest that thorough training of young scientists in careful and critical reading of research papers would help. An excellent means to this end is an active journals club, where each member regularly discusses a paper in depth. Second, in the special case of university scientists, the perception that output of publications is virtually the sole criterion for promotion needs to be modified, while promotions committees should put increasing weight on the teaching dossier and administrative competence; teaching and administrative duties require as much skill and time as laboratory research, and although they reduce research output they do not detract from its quality. Third, grant-making bodies should pay attention to modification of the role of the progress report as a step towards preventing the spread of this new disease.

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1. Collins, E. *Nature* 324, 197 (1986).

2. Locke, R. *Nature* 324, 401 (1986).

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5. Braunwald, E. *Nature* 325, 215–216 (1987).

SIR—The commentaries by Stewart and Feder and by Braunwald (*Nature* 325, 207–216; 1987) have drawn attention to an increasingly painful issue in science. Darsee's apparently fraudulent work is not an isolated case.

The integrity of science has always rested on two pillars: the honesty of the individual scientist, and the reproducibility of the research findings. This attitude has served science well in the past, partly because the number of researchers were few and because they published only when substantial data had accumulated.

In the postwar era, the increased infusion of funds into the scientific establishment and the training of large numbers of scientists may have overwhelmed this old-fashioned system. The products of science have also entered the consumer market and, as a result, have become big business. The scientific entrepreneur is now competing with major industries in raising money for his ventures. Science has also become part of government legislation. The environmental concerns of the 1960s have spawned a major industry devoted entirely to the evaluation of new and old chemicals. The complexity of the technologies involved in all aspects of this 'new' science has resulted in multiple investigators pooling resources to answer questions that were previously unanswerable or were only partly answered because no individual had all the skills required to deal with these complex problems. Finally, with the large number of trained researchers competing for funds that wax and wane with political fortunes in Washington, the pressure to publish in order to succeed has increased.

A hundred years ago, it would have seemed impossible for a researcher to publish 50 papers per year, and yet today many scientists are not only capable of maintaining this pace year after year but also of exceeding it. Who can possibly replicate the flood of papers published by the journals which, having become part of the science business, encourage an ever greater pace?

It is not surprising that under these circumstances more and more cases of fraud are discovered. The pressures are simply too great, and the temptation to become a sharer of this \$100,000 million yearly enterprise irresistible. Are we to assume that the revealed cases of fraud represent the fringes of science, or do they represent the tip of the iceberg? Can we go on assuming that trusting the integrity of the individual scientist is sufficient to police our ranks and prevent fraud in the future? It is hard to believe that with so much money at stake, any other profession would act on trust alone.

This historical development of science

could be compared with the developments in many other fields during the same period. Thus over the years banking has evolved from a state where transactions were conducted between people who knew each other in small communities, and with a handshake sealed a contract. Business deals were agreed upon in a similar way. As communities grew larger, it became necessary to commit contracts to paper and eventually a complex set of rules has evolved governing these transactions.

One consequence of this evolution is the necessity to ensure that ethical procedures are followed in the transaction of the business. The mechanism ensuring this is the auditing of books by independent professionals who are trusted by the business community to provide an unbiased evaluation of the business practices of the parties involved in the transaction. This has become an accepted practice because, without it, the same temptations that we are beginning to see in science would destroy the credibility of business and bankers alike.

Before science loses its credibility and scientists become regarded as untrustworthy members of society not meriting the financial support needed to carry out research, it is necessary to establish data-auditing procedures that will ensure that fraudulent practices are minimized and revealed early. The procedures for data auditing will have to be non-obtrusive so that scientists can carry out their work without fear of unnecessary interruptions. In fact, an entirely new field of science data-audit will have to be developed in order to make the supervision of the vast research enterprise possible. This may be perceived as yet another unnecessary piece of bureaucracy, but we feel that without such a safeguard scientists will not be able to maintain their reputation for honesty and integrity in the face of ever increasing fraudulent practices by a minority that cannot be weeded out before its entry into the field.

In the long run, just as the banks accept auditing as part of their normal activities and because of this safeguard have earned a reputation as trustworthy institutions, scientists will have to accept data-auditing as a normal procedure, and will use data auditing techniques to reassure the funding agencies and society in general that they have learned to police their ranks in a mature and professional manner.

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Benzene's electron structure

A new calculation shows that Kekulé may have made a good model of benzene, but also fails to resolve the question whether valence bonds are better than molecular orbitals (or vice versa).

PETER A. SCHULTZ and Richard P. Messmer are at it again: that is the simplest thing to say of the paper the two theoretical chemists from the Department of Physics at the University of Pennsylvania at Philadelphia have now published on the electronic structure of benzene (*Phys. Rev. Lett.* **58**, 2416; 1987). Under the title "Are there π bonds in benzene?", the two authors say that the answer is probably negative, and that the best description of the benzene molecule is probably based on an oscillating ("resonating", *vide* Pauling) blend of the two obvious equivalent patterns of alternating double bonds originally proposed by Kekulé in 1872.

Some recent parochial history of this tale is relevant. In October last year, Cooper, Gerratt and Raimondi from the Universities of Liverpool, Bristol and Milan, published (*Nature* **323**, 699; 1986) an account of the electronic structure of benzene, now described by Schultz and Messmer as the "prototypic aromatic molecule". Cooper *et al.* concluded that the now-standard textbook description of the benzene molecule as a symmetrical and essentially static structure is probably incorrect. In particular, they concluded, the electrons supposed, in the usual textbook description, to form two hexagonally shaped rings above and below the otherwise planar structure of the molecule (the famous π electrons) are probably more localized on the atomic nuclei than the standard view would allow.

A few weeks later, Schultz and Messmer published (*Phys. Rev. Lett.* **57**, 2653; 1986) an equally interesting reappraisal of the electronic structure of a molecule, in that case C_2F_2 . Again it seems that a valence-bond calculation of the structure of the molecule had shown that textbook expectations are not fulfilled. Acknowledging that the two pieces of work were demonstrably independent of each other, *Nature* nevertheless cheekily chided the second group of authors for having claimed their work to be the "first" of its kind (*Nature* **324**, 599; 1986).

The latest development is more mystifying, given the history, for the latest paper refers neither to the original paper by Cooper *et al.* nor to the teasing commentary on the affair that appeared afterwards in *Nature* (but which Schultz and Messmer may well have judged beneath contempt). That, however, has an innocent explanation; surprisingly, it turns out that the latest paper from Schultz and Messmer, in

the issue of *Phys. Rev. Lett.* for 8 June, had been submitted for publication on 2 September last year, six weeks before the original paper by Cooper *et al.* appeared in *Nature*. Why the paper has been hanging about all this time is a matter for the authors and the journal; otherwise the oldest paper in the issue of 8 June is dated early in February this year. But, in the circumstances, may not Cooper *et al.* have rated a "note added in proof", a common convention in pure physics journals having to survive the flood of preprints that threatens to overwhelm them?

The importance, such as it is, of this tale is as an illustration of how even the modern literature is not nearly as well-ordered as it might be. References to other people's work, conventionally the means by which authors acknowledge those (giants and otherwise) on whose shoulders they have stood, are also convenient cross-references between parallel developments. Perhaps some of the energy now being spent on making the literature electronic should be used, in passing almost, to solve that problem.

None of this is intended to distract, let alone detract, from what Schultz and Messmer (not to mention Cooper *et al.*) have done. The classical problem of benzene and other aromatic compounds is understanding why carbon atoms, which usually form four tetrahedrally arranged bonds with other atoms, should sometimes form trigonal bonds with added stability (as in benzene, where each carbon is bonded to one hydrogen and the two neighbouring carbon atoms in a hexagonal ring). Rational explanation came only in the late 1920s, with the recognition that the spherically symmetrical s and polar p orbitals of the second quantum level are energetically degenerate and, with the neglect of inter-electron interactions, may be rearranged into a set of polar orbitals arranged trigonally in a plane, with a symmetrical (strictly, an anti-symmetrical) polar orbital, the famous π , at right angles to the plane.

The neatest feature of this argument is that it provides an easy way of calculating something. On the assumption that a benzene molecule is held in place by the within-the-plane bonds between carbon atoms (called σ bonds), it is a fair question to ask what extra binding energy arises from the interaction between the ring of six dumbbell-shaped orbiting electrons at the vertices of a benzene ring. Naturally, the

simplest calculations are grossly approximate; the simplest of all is to assume that electrons in the molecule as a whole are adequately described by linear combinations of the atomic π orbitals (with complex coefficients to allow them to seem orthogonal to each other). But there are many other tricks that can be played, using the calculated wave functions of electrons in the force-field of a hexagonal charged structure, for example.

The other way of dealing with these problems is more intuitively appealing, based as it is on the arm-waving that passed for theoretical chemistry before quantum mechanics arrived. Given the valency of atoms and the geometrical propensities of their bonding patterns, it is relatively easy to construct all possible structures. The trick, easily translated into wave mechanics language, is then to suppose that the true state, in the sense of quantum mechanics, is a superposition of all possible states. It is like psychoanalysis, where one looks for the "baby" or the "destructive infant" part of a person.

In simple cases, the valence-bond approach allows calculations to be carried through, for the hydrogen molecule for example. For molecules as complicated as benzene, the calculations have only recently become realistically possible with the arrival of Cray computers and the like, but the alternative valence-bond structures that matter are those originally described by Kekulé.

The two calculations now described (Cooper *et al.* and Schultz and Messmer) are complementary. The first showed that electrons do tend to be more localized on the atoms at hexagonal vertices than the simple picture of entirely mobile π electrons suggests. Schultz and Messmer have the more detailed pictures. Their particular contribution is their concept of what they call the "bent- Ω " bond.

But Schultz and Messmer raise one potentially contentious issue by saying that they believe valence-bond calculations to "have a significant advantage" over other approximations in retaining "an interpretability" with a direct relationship to the "almost universally accepted concepts of structural chemistry". But all these methods of calculation can only be approximations to the same truth — and Mulliken's Chicago school, with its distinctive view of structural chemistry, would have hotly disputed the conclusion.

John Maddox

Human genetics

Abducted orphans identified by grandpaternity testing

Jared M. Diamond

USE of genetic methods to resolve cases of disputed paternity has been accepted legal practice for many years. Paternity testing, however, exemplifies a potentially much broader problem: how to decide whether person X could not, might, or almost surely does stand in some postulated relationship to persons $Y_1, Y_2, Y_3, \dots$. Stimulated by the recently emerging need for grandpaternity testing, Ana Maria Di Lonardo, Mary-Claire King, Cristian Orrego and others have recently developed methods¹⁻⁴ and theory^{1,5,6} of extended relationship testing.

The stimulus for this work was the disappearance of at least 9,000 Argentinians between 1975 and 1983, abducted by special units of the then-ruling military and police who claimed to be defending national security and honour. Among these 'desaparecidos' were at least 195 children, most of them born during detention to mothers pregnant when abducted (see photo), but some abducted with their parents or alone. Most adult desaparecidos have not reappeared and are known or presumed to be dead, but a human-rights group, the Grandmothers of the Plaza de Mayo, has located dozens of children believed to be survivors of the missing 195. In some cases, the child is living with military couples who claim to be the biological parents but who are instead suspected of being kidnappers (for example, because the military mother was not observed to be pregnant before the baby's sudden arrival). Some of the military fathers are thought to have been involved in the murder of the child's real parents. How can the true identity of such a child be established when the presumed

parents are dead but some or all of the presumed grandparents are still alive?

Di Lonardo and her colleagues use genetic codominant markers, such that genotypes are equivalent to phenotypes. Most informative are loci with many alleles, with rare alleles, or two or more loci closely linked on the same chromosome so that allele combinations (haplotypes) are inherited as a unit. In practice, the markers chosen are ones that can be determined in blood samples: human leukocyte antigens (HLA), blood groups, red cell enzymes, plasma proteins and DNA polymorphisms. Samples are drawn from the putative grandparents and (under court order) the disputed child; the military 'parents' legally can and do refuse to be sampled.

If the child has an allele absent in all four putative grandparents, grandpaternity can be excluded. Conversely, if the child's alleles are carried by the putative grandparents, one can calculate the likelihood that the child shares the alleles with the putative grandparents by inheritance, and the likelihood that the alleles are shared only by chance. The probability that the putative grandparents are the actual ones increases with the ratio of the former probability to the latter. The rarer the shared alleles in the general population, the more certain is the identification of grandpaternity.

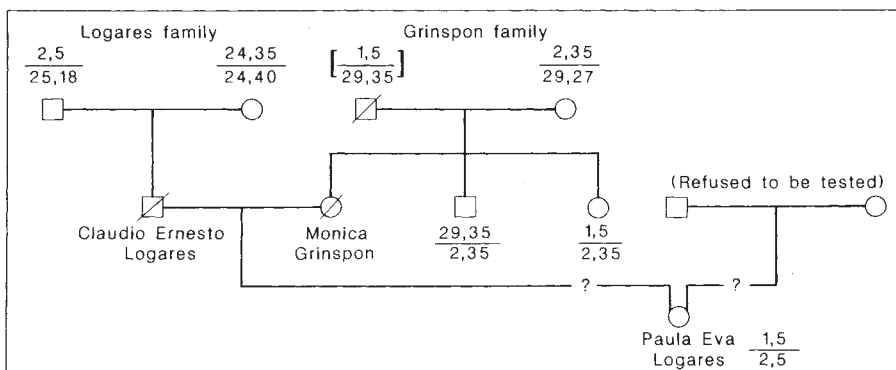
Each case studied turns out to be a new drama, because of the differing alleles, differing human tragedies and differing living putative relatives involved. If one or more putative grandparents is dead, that grandparent can sometimes be 'genetically reconstructed' from putative aunts,



Case 195, Liliana Pereyra (age 21). In 1977 Liliana was abducted when she was 5 months' pregnant. She was kept alive in a torture centre (ESMA Military Academy) until she gave birth to a boy in February 1978, whereupon she was killed by a shotgun blast to the head from close quarters. Her skeleton, found in a cemetery where other desaparecidos were buried, was identified in 1985. Her son has not been found and may have been kidnapped. (Information from the Grandmothers of the Plaza de Mayo.)

uncles and siblings of the disputed child. As an example, consider the pedigree illustrated in the family tree. An eight-year old girl lived with a policeman and his wife, who alleged her to be their natural daughter, but the birth certificate appeared fraudulent. The girl was instead suspected to be Paula Eva Logares, abducted at the age of 2 on 18 May 1978 with her parents Claudio Ernesto Logares and Monica Grinspon, who were never seen again. In 1984 three of the four putative grandparents were alive, and their HLA-A,B haplotypes (and blood groups) were determined (see legend of family tree for details). Monica Grinspon's father was dead, but his HLA haplotype could be reconstructed as (1,5)/(29,35) because Monica's surviving brother and sister had the haplotypes (29,35)/(2,35) and (1,5)/(2,35), respectively. As her mother had (2,35)/(29,27), (1,5) and (29,35) must have been transmitted to her brother and sister from her father. The alleged parents refused to be tested.

The child's haplotype was (1,5)/(2,5), compatible with (1,5) having been inherited from Monica's father and (2,5) from Claudio's father. On the one hand, the probability of each grandparental haplotype being transmitted to a grandchild is 0.25: four haplotypes are present in each pair of grandparents, and one of the four is inherited by the grandchild. Thus, the probability that the child and putative



Two competing pedigrees of Paula Eva Logares, with HLA-A,B haplotypes where available. Squares, males; circles, females; slashes, deceased persons; numbers, HLA antigens for blood groups A, B on the two chromosomes of each individual; brackets, reconstructed haplotype of Monica Grinspon's deceased father. Paula was reared by a policeman and his wife as their daughter, but HLA makes it more than 99.9 per cent certain that she is the grandchild of the Logares and Grinspon families. (From ref. 1 and information supplied by Mary-Claire King.)

grandparents would share these haplotypes if related is $(0.25)(0.25) = 0.0625$. On the other hand, the frequencies of both haplotypes are low in Argentina: 0.002 and 0.036 for haplotypes (1,5) and (2,5), respectively. It is very unlikely that an Argentinian child chosen at random would carry the combination of those two haplotypes, and the actual probability is $2(0.002)(0.036) = 1.44 \times 10^{-4}$. (The factor two arises because a child born to parents drawn at random could have obtained the first haplotype from the mother and the second from the father, or vice versa.)

The probability of grandpaternity for the Logares/Grinspon families is therefore $0.0625/(0.0625 + 0.0001) = 0.999$. That is, HLA alone establishes with 99.9 per cent certainty, and blood groups make it even more certain, that the child is Paula Eva Logares. By court order she moved to her grandparents' house, where she went directly to her former room and asked for a doll that she had last seen at the age of two.

Di Lonardo, King and their co-workers have so far applied genetic methods to the case of 39 Argentinian child desaparecidos. HLA turns out to be the most informative marker and, together with blood groups, has sufficed to resolve all cases. It was initially feared that the decision as to whether to transfer a child from military 'parents' who were kidnappers but had nevertheless reared the child for years would be agonizing. In practice, the transferred children became integrated into their biological families with minimal trauma, perhaps in part because murderers made poor parents. (Paula Eva Logares has been increasingly resistant to the court-ordered visits of the alleged parents.) In four cases where innocent adoptive parents reared a child in good faith (for example, because the desperate mother had handed her baby to a passerby just before being abducted), the situation has been resolved amicably by joint custody or visiting arrangements between biological and adoptive families.

The project of Di Lonardo and colleagues¹ is now expanding in three directions. First, the Grandmothers of the Plaza de Mayo are assembling a genetic databank on grandparents whose grandchildren are still missing, and on children who suspect that they are desaparecidos but whose grandparents have yet to be identified. This databank may permit grandchildren to be identified even after some grandparents have died.

Second, although DNA methods have yet to be required in the 39 Argentinian cases analysed so far, they will surely be needed eventually (for example, if only one grandparent is still alive). Hence, King and Orrego are developing grandpaternity techniques based on DNA polymorphisms such as Jeffreys' minisatellite

sequences or mitochondrial DNA^{7,8}. The latter, being transmitted only by the mother and hence identically shared by siblings, will be useful in cases where the putative mother is dead but putative maternal aunts or uncles survive.

Finally, the extended relationship-testing protocol of Di Lonardo *et al.* is relevant to other cases of disputed identity, such as families separated by war or natural disasters, unidentified dead bodies and putative impersonators⁹. □

Embryology

We have a morphogen!

J. M. W. Slack

THE first morphogen has been identified and it is retinoic acid. Of course nothing in science is really certain, so perhaps I should say that the article by Thaller and Eichele on page 625 of this issue¹ completes a strong *prima facie* case for the identity of the first morphogen. So what is a morphogen and why is this work so important?

In embryology there are often cases where a uniform sheet of similar cells develops into a series of structures whose position and polarity is controlled by an organizing centre. Morphogens are the hypothetical signal substances emitted from these centres, the idea being that cells respond to different concentrations by adopting different pathways of differentiation and so produce the characteristic anatomy of the organ concerned. A morphogen is thus a particular kind of inducing factor characterized by the evocation of different cellular behaviours at different concentrations.

This particular story really started in 1968 when Saunders and Gasseling described² a zone at the posterior margin of the chick embryo limb bud that was able to induce an extra set of limb structures when grafted to the anterior side. This was later called the zone of polarizing activity (ZPA). In embryonic animal limbs, 'anterior' means the side bearing the thumb or big toe, and 'posterior' the side bearing the little finger or little toe. The digits of the chick wing are called 2,3 and 4 from anterior to posterior. If a ZPA is grafted to the anterior side of the bud then a second set of digits can be induced which lie in a relation of mirror symmetry to the original set, that is, graft ZPA-4',3',2',2,3,4-host ZPA. Tickle, Summerbell and Wolpert in 1975 presented³ a series of such grafting experiments and analysed the results in terms of a model in which the ZPA emitted a diffusible morphogen that was destroyed by the competent tissue, thus forming a steady-state exponential concentration gradient. They proposed that in normal

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development the pattern of digits, and indeed all the other structures in the limb, arise as a result of threshold responses by the cells at the appropriate morphogen concentrations.

Several substances were tested by absorbing them onto slow-release media and grafting into the anterior side of the wing bud, but none of these experiments was successful. It was suspected, however, that the signal travelled from cell to cell through the pore-arrays called gap junctions, and so retinoic acid, an agent that could inhibit gap-junction communication, was eventually tested. To everyone's surprise, local application of retinoic acid to the anterior side of the bud produced duplications exactly similar to those produced by ZPA grafts⁴. Subsequent study of the distribution of applied retinoic acid showed that an exponential concentration gradient is indeed set up and approximate concentration thresholds for the induction of digits 2, 3 and 4 were deduced^{5,6}.

There was some scepticism about the possibility that retinoic acid was the endogenous morphogen, partly because the molecule cannot be synthesized *de novo* by animal cells and partly because it has many other biological effects which made an indirect mechanism seem likely. But it was of obvious interest to know whether the limb bud contains retinoic acid; whether there is a graded distribution from posterior to anterior; and whether the levels correspond to the known induction thresholds in response to applied retinoic acid. Thaller and Eichele now report¹ that the answer to all three questions is 'yes'.

As usual in biochemical embryology, measurements such as these are much harder than they sound because of the tiny quantities of tissue and the impossibility of radiolabelling the endogenous retinoic acid to a high, known specific activity. Interested readers will doubtless study Thaller and Eichele's paper, so I shall simply draw attention to their heroic achievement in dissecting and bisecting

5,536 limb buds to measure the antero-posterior distribution. The authors also show that the limb bud contains relatively high levels of retinol that can serve as a precursor for retinoic acid and keep the source going.

Thus, retinoic acid can induce extra structures and is present in the appropriate amounts and spatial distribution to be responsible for inducing the normal pattern of structures. There are about 10^5 molecules of cytoplasmic retinoic-acid binding proteins per limb-bud cell⁷, and binding to this protein is often assumed to be the first step in the action of retinoic acid. Although the formal proof of its identity as a morphogen might seem beyond dispute, there are still some major uncertainties. It could be, for example, that increasing retinoic acid concentration is the first step in the response, so application of retinoic acid could bypass the normal morphogen, which is some other diffusible factor. More fundamentally, exactly what is being induced? If it is literally true that the sequence of digits is induced by the gradient then there must be two threshold responses for each digit and the cells would become cartilage between the thresholds and fibroblastic outside them. However, there is some evidence^{8,9} that a set of cartilage elements can be formed anyway, without any morphogen, and this would leave as the only function of the signal the conversion of an exactly periodic pattern to a 'non-equivalent' one in which the individual elements acquire, in later development, the lumps and bumps that enable anatomists to distinguish and name them. My own suspicion is that this sort of phenomenon, although an interesting problem, may not be as biologically fundamental as those 'morphogen-gradient' processes in which a set of structures composed of different sorts of cells are formed as, for example, in the establishment of the primary body plan in vertebrates.

It is probable, as Thaller and Eichele admit, that acceptance of the role of retinoic acid as a morphogen will not be widespread until the biochemical mechanism of its action has been elucidated. This is all the more interesting because retinoic acid has other interesting developmental effects, among which are pattern alterations in regenerating limbs¹⁰, promotion of differentiation in embryonal carcinoma cells¹¹ and promotion of mucinous metaplasia in squamous epithelia¹².

Finally, it may be of interest to compare retinoic acid with some other possible morphogens which have been described recently. Three of them are also low-molecular-mass hydrophobic substances which, like retinoic acid, may be able to pass from cell to cell without gap junctions or specialized receptors. These molecules are DIF¹³, which induces stalk cells in the slime mold, and the inhibitors of head and

foot regeneration in *Hydra*¹⁴, which specifically retard regeneration of the appropriate extremity. But the *Hydra* head and foot activators are both peptides, and the best candidates for mesoderm-inducing agents in amphibian embryos are fibroblast growth factor¹⁵ and a small protein¹⁶, both of which are likely to require cell surface receptors to exert their effects. Evidently the once-popular idea that all morphogenetic signalling systems are the same¹⁷ is not being borne out by the results. □

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Solar System

Growing planetary atmospheres

Philip Campbell

THERE cannot be many scientific disciplines so afflicted by a lack of direct observational evidence as the study of the origin and early evolution of planetary atmospheres. So it might have seemed unnecessarily perverse of J.B. Pollack (NASA Ames Research Center), one of the organizers of a recent meeting*, to challenge participants at the outset to restrict themselves to theories that make unique or 'strong' predictions. The fact that the participants did not immediately pack their bags perhaps reflects a sense of recent progress in that some hypotheses have been eliminated, and others have been placed on a firmer quantitative footing.

Looking outside our Solar System, observers can but hope that star-formation regions elsewhere in our Galaxy reproduce conditions within the pre-solar nebula. Observations of the Taurus-Auriga complex, reviewed by A.P. Boss (Carnegie Institution, Washington) and colleagues, indicate that fragmentation of clouds to protostellar clumps of several solar masses occurs readily in such environments and that this arises, with the help of magnetic fields coupled to the ionized gas, without the generation of problematically large angular momentum. Indeed, three-dimensional simulations of binary system formation by Boss indicate that a low specific angular momentum is essential at this stage to avoid further fragmentation.

Outward transport of angular momentum is also an important means of preventing such fragmentation, but this must be reconciled with the need to transfer mass inwards to feed the proto-Sun. The growth

of an accretion disk is one conventional solution, but recent estimations of the properties of dust grains suggest that the vertical turbulent convection required for radial mixing in most models would not after all have been widespread, whereas dynamical calculations indicate that such disks would be less viscous than hitherto believed. According to Boss, however, gravitational torques from asymmetrical structures such as bars and spiral waves might have accomplished the required transport of angular momentum.

Of the two principal theories for the ensuing formation of planets (reviewed by R. Greenberg, Univ. Arizona) one, involving giant gaseous protoplanets, is now unpopular. Instead, despite the significant uncertainties involved, the idea that solids — icy or rocky — aggregated to form planetesimals which then accreted to form the inner planets and the cores of the outer planets, now holds sway and provides the basis of many of the planetary models presented at the meeting. A traditional problem of such a model, that it predicts growth times for Uranus and Neptune in excess of the age of the Solar System, can be resolved by invoking run-away accretion of gas at the end of the growth of the rocky interiors of these two planets (Pollack). The terrestrial planets — Mercury, Venus, Earth and Mars — would have accreted over a period of about 100 million years in such a model.

Any attempt to model the growth of a planet must explain the distributions of chemical elements and their isotopes that are observed today. Furthermore, Venus, Earth and Mars are thought to have formed under conditions sufficiently similar that a model of one must be relevant to the others.

* *The Origin and Evolution of Planetary and Satellite Atmospheres* Tucson, Arizona, 10–14 March 1987.

Among the most stringent constraints are the abundances of rare-gas isotopes, reviewed by M. Ozima (Univ. Tokyo). For instance, it is well established from the relative abundance of radiogenic ^{40}Ar that a period of intense outgassing from the Earth's mantle occurred within the first billion (10^9) years of the planet's history. But it emerged at the meeting that the significance of two other possibly crucial processes relevant to primordial atmospheres — one catastrophic but speculative, the other slow but sure — has become quantifiable only during the past year or so.

The first of these processes relates to impacting planetesimals. There is every reason to suppose that such bodies, particularly during the late stages of accretion, contained volatiles such as water. Laboratory experiments in which serpentine (a hydrated magnesium silicate) is shocked by projectiles from a gun to pressures as extreme as 60 GPa suggest that, by the time the Earth had grown to 50 per cent of its present radius, impact pressures would be sufficient to remove water and other volatiles completely from incoming planetesimals (T.J. Ahrens, Caltech). The Earth's primordial atmosphere could have been generated by these means as much as by volcanic outgassing.

But, as M.A. Lange (Alfred-Wegener-Institut, Bremerhaven) and Ahrens described, a primordial atmosphere should also have been eroded by impacts. As much as 45 per cent of the kinetic energy of incoming kilometre-sized planetesimals could have been transferred to crustal ejecta and thence to the atmosphere via blast waves, one such impact possibly removing over a millionth of the atmospheric mass. But after including such calculations in accretion models and considering all the uncertainties, it is still impossible to judge whether planetesimal

impacts were net deliverers or removers of the primordial terrestrial atmosphere.

Another process crucial to atmospheric evolution is hydrodynamic escape, whereby trace gases are dragged out of an atmosphere by a light, dominant species, such as hydrogen on the early Earth. It remains an open question as to how much hydrogen enveloped the Earth at the time of its formation although, as H. Wanke (Max Planck Institut für Chemie, Mainz) emphasized, reactions between iron and water (both abundant then) should have generated large quantities. But it is only in the past year, as reviewed by D. M. Hunten (Univ. Arizona), that the process of hydrodynamic escape has been placed on a sufficient theoretical footing for it to be readily incorporated into planetary models.

A crucial aspect of this escape process is that it is very sensitive to atomic mass, providing a means of fractionating rare-gas isotopes — an attribute exploited in a new model of terrestrial planetary evolution by R.O. Pepin (Univ. Minnesota). Incorporating as sources of primordial noble gases the solar nebula and the more 'exotic' products of a pre-solar supernova, and invoking adsorption on nebular grains and hydrodynamic escape as means of fractionating both elements (by the former) and isotopes (by both), Pepin could produce an excellent fit to the terrestrial and martian abundances of primordial isotopes of xenon and krypton. (The abundances on Venus are poorly determined.) In such a model all the uncertainties associated with planetesimal accretion apply, while some special pleading seems necessary to account for abundances of a few of the other rare-gas isotopes. Behind this and other models there is also a debate about the extreme-ultraviolet flux from the early Sun. A strong flux of more than 20 times the present value would have been needed for more than 100 million years to drive off the hydrogen and provide the required fractionation, and significant questions remain as to how the emission of such radiation, not to mention the opacity of the nebular gas and dust, did in fact evolve.

Several speakers at the meeting stressed the need, first highlighted by T. Matsui and Y. Abe last year (*Nature* 319, 303; and 322, 526; 1986) to consider, when modelling the late stages of planetary accretion, the thermal blanketing that would have arisen from steam in the primordial atmosphere. Detailed calculations of water and energy budgets (K. Zahnle *et al.*, NASA Ames) support the idea that a magma ocean should have formed under a runaway-greenhouse atmosphere generated by the time the Earth had grown to one half its final radius. Zahnle emphasized the role of the melt, not only as a reservoir of water during accretion but also as a source of sudden degassing when,

during the subsequent cooling, the magma ocean reached the solidus temperature and the solubility of water decreased.

Several times the mass of the present-day oceans could have been lost by hydrodynamic escape while the Earth accreted. The analysis of the final condensation of water is complicated by non-ideal behaviour of gases in an atmospheric regime close to the critical point of water, as highlighted by Matsui and Abe (Univ. Tokyo), but their one-dimensional radiative-convective model suggests that a hot proto-ocean would have rained out rapidly during the final stages of accretion.

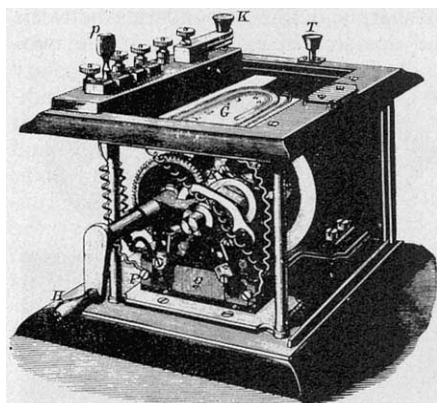
Greenhouses and bolides must also have played a role on Mars and Venus. The presence of gullies and valley networks on Mars implies that conditions once permitted liquid water to flow on its surface. Paradoxically, the early Sun (during its first billion years or so) was about 25–30 per cent fainter than at present. But J. F. Kasting and O. B. Toon (NASA Ames) calculate that 5 bar of atmospheric CO_2 would have warmed the surface above the freezing point of water. Such an abundance would have required a continuous source, as chemical weathering would have removed atmospheric CO_2 to the crust. Large-scale volcanism seems to be one of the few possibilities, such recycling ceasing once the planet had cooled sufficiently.

The present-day role of the greenhouse effect on Venus is well established, but the history of water on that planet remains a tantalizing issue. The present-day abundance is 100,000 times smaller than that on Earth, whereas the deuterium-hydrogen (D/H) ratio in the atmosphere of Venus, interpreted in the light of known escape mechanisms, implies that at least 100 times more water was initially present. Kasting and Toon showed that liquid water might once have existed there if the net radiative effect of clouds (whose behaviour is currently unquantifiable) was to cool the planet.

But D. H. Grinspoon and J. S. Lewis (Univ. Arizona) offered a provocative new interpretation of the venusian D/H ratio. Dynamical calculations now suggest that active cometary nuclei or their fragments (as well as other bolides) should encounter planets at irregular intervals throughout the history of the Solar System. Given the water content of such bodies, the atmospheric water abundance on Venus should have oscillated between 100 and 400 parts per million — within the uncertainties of current estimates. According to this hypothesis, 99 per cent of the water now on Venus is of cometary origin. If this is indeed the case, then the enhanced D/H ratio in the present atmosphere may reveal little about the history of water on that planet. □

Philip Campbell is Physical Sciences Editor of *Nature*.

100 years ago



By the use of the secohmmeter it is now possible not merely to measure the coefficients of self and mutual induction absolutely, but also to secure the same high degree of sensibility with comparison tests completed during the growth or dying away of a current.
From *Nature* 36, 129; 9 June 1887.

Molecular genetics

A genetic switch in *Drosophila* morphogenesis

Miranda Robertson

DROSOPHILA developmental genetics have been much advertised in these pages as the most promising route to an understanding of the molecular biology of morphogenesis, even if we do not yet — as the unwary might have concluded from the titles of two recent articles^{1,2} — know how to make a fruitfly. Recent developments in David Hogness's laboratory may, however, have brought this prospect a quantum jump closer to realization. The relevant experiments were reported at a recent meeting* by Mark Krasnow (Stanford), whose achievement, briefly, is to have demonstrated, by transfecting cultured cells with cloned DNA, the existence of predicted but unproven regulatory interactions between three genes known to have a fundamental role in the specification of the body plan of the fly.

The prevailing picture

The interactions that Krasnow investigated were between two major classes of gene believed to contribute to two separate but coordinated processes in the specification of the body plan. The first class comprises the segmentation genes, which determine segment boundaries. Mutations in these genes result in the deletion of specific subsets of segments.

The second class of genes comprises the homoeotic selector genes whose differential activation in the different segments determines the individual character of each segment, ensuring, for example, that legs grow from the thoracic segments and antennae on the head. Mutations in these genes transform one segment into another (and can indeed, notoriously, cause legs to grow from the head instead of antennae). Several lines of evidence suggest that the segmentation genes contribute to the specification of segment identity by determining both the level and the combination of selector gene products expressed in each segment — the interactions of the selector genes themselves being responsible for the final determination of segment character.

The simplest way to envisage such interactions is to imagine that the products of the segmentation genes directly regulate the transcription of the selector genes; and that the products of the selector genes regulate one another's transcription (and directly or indirectly that of other genes).

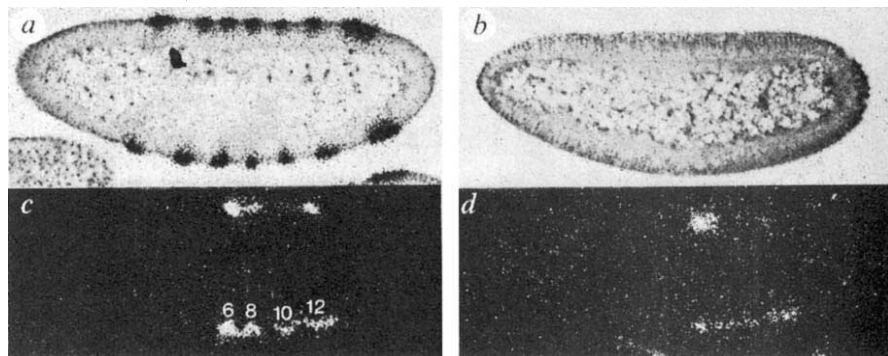
Support for this notion has been drawn from two sources. First, mutations in one

segmentation or selector gene can be shown to affect the expression of others. Second, the segmentation and selector genes contain sequences with homology to two known sequence motifs of DNA-binding regulatory proteins. Two of the segmentation genes (*Krüppel* and *hunchback*) have homology to the metal-binding finger motif of the vertebrate TFIIIA proteins (Herbert Jaekle, Tübingen; refs 3, 4); and a third (*fushi tarazu*, or *ftz*) shares with the homoeotic selector genes the canonical homoeobox domain, part of which has some homology with the helix-turn-helix motif of the bacterial gene-regulatory proteins.

The helix-turn-helix homology of the *Drosophila* homoeobox domains is, however, not strong, and the only existing evidence that they specifically bind regulatory sequences in DNA is highly suggestive rather than definitive⁵. The new work of Krasnow and of Phil Beachy in Hogness's laboratory provides the first direct evidence that products of these genes bind specifically to DNA and regulate transcription.

The segmental switch

The three genes Krasnow used in these experiments were the segmentation gene *ftz*, which is expressed in all even-numbered segments; and two major homoeotic selector genes, *Antennapedia* (*Antp*) and *Ultrabithorax* (*Ubx*). *Antp* specifies principally the structures of the anterior thorax, *Ubx* more posterior thoracic structures, the segmental expression of each overlapping with that of the other. All three genes contain a homoeobox.



Distribution of transcripts of *ftz* and *Ubx* in wild-type and *ftz*⁻ mutant *Drosophila* embryos at the onset of gastrulation. *a*, Periodic distribution of the *ftz* transcript in a normal embryo; *b*, a mutant embryo not expressing *ftz*; *c*, discrete peaks of *Ubx* transcription in segments 6, 8, 10 and 12 in a normal embryo; *d*, abnormal distribution of *Ubx* transcripts in a *ftz*⁻ embryo. The examination of the pattern of transcription of various selector genes in embryos deficient in segmentation genes has helped to show how these genes act by influencing one another's expression. (From Fig. 3 of ref. 6.)

The interactions of these genes were tested using four plasmids, two expressing *ftz*- and *Ubx*-coding sequences, respectively, under a powerful promoter, and the other two containing the standard chloramphenicol acetyltransferase (CAT) reporter gene under the control of either an *Antp* or a *Ubx* promoter. The experiments with *ftz* were done in collaboration with Gary Winston and Matt Scott at the University of Colorado. By cotransfecting *Drosophila* tissue-culture cells with different combinations of the plasmid constructs and measuring CAT activity, they were able to show in transient-expression assays that the *ftz* gene product activates both *Ubx* and to a lesser extent *Antp*, and that the *Ubx* product activates its own expression and represses *Antp* (an effect that is measurable because *Antp* has some constitutive activity in the tissue-culture cells).

Taken at face value these experiments are remarkable for two reasons. First, the interactions are as predicted by the developmental genetics of the fruitfly and more recently by direct analysis of the patterns of expression of the three genes both in wild-type and in *ftz*⁻ *Drosophila* embryos (ref. 6; see figure). Thus in the absence of *ftz*, the pattern of expression of both *Ubx* and *Antp* is abnormal, while the relationship of *Ubx* and *Antp* is uncannily reminiscent of that between λ repressor and *cro* (see ref. 7 for an authoritative account, or part I of this report⁶ in News and Views last week for a brief recapitulation). *Ubx*, once activated, is normally expressed throughout development in a pattern that changes with time; and it has been suggested that the maintenance of *Ubx* expression may depend in part on an autoregulatory mechanism. In the absence of *Ubx*, *Antp* is expressed in segments from which it is normally absent; and during normal embryogenesis, overlapping distributions of the two gene products gradually resolve into a pattern of sharply defined boundaries between the domains of expression of the two.

*The Spring Meeting of the Genetics Institute was held in Cologne on 3–6 March 1987.

This pattern could imaginably be brought about by competitive interactions operating a switch whose position would be determined by the relative concentrations of the *Ubx* and *Antp* products — which are in turn determined by the segmentation genes.

The second remarkable point about the experiments is that they worked at all. *Ubx* is an extremely large and complex locus which Kornfeld and Hogness have shown gives rise by differential splicing to at least five different proteins whose correct expression normally depends upon a 40-kilobase 5' control region called *bxd*. Krasnow was therefore surprised that he was able to get intelligible results from a simplified system in culture. Indeed, so far he has investigated only one of the five *Ubx* proteins, *UBX Ib*, specific binding sites for which have been identified in Hogness's laboratory by Phil Beachy, with Krasnow and Elizabeth Gavis, by footprinting *in vitro*. These sites, which were used in Krasnow's CAT plasmids, consist of TAA repeats of which a minimum of four are required to bind the *UBX* protein. Two of these repeated sequences occur downstream of the start sites of both *Ubx* and *Antp*, and *Antp* also has two upstream — an unusual arrangement that in the case of *Ubx* has been shown to be conserved in two distantly related *Drosophila* species. With the availability of a functional assay for the interactions of these developmental regulatory proteins with DNA, the activities of the other *Ubx* products, as well as the products of other segmentation and selector genes, and the significance of the unusual organization of the *UBX Ib* binding sites, are open to investigation.

Regulatory interactions

Indeed, the success of these experiments opens up the possibility of directly testing the importance of interactions between regulatory proteins, and perhaps of DNA looping, in gene regulation in development. For example, interactions between regulatory proteins that bind to different parts the huge *bxd* control region may provide an explanation for some highly suggestive observations, made by Steve Helfand (now at Yale University, then in Hogness's laboratory), that as increasingly large deletions are made from the 5' end of the *bxd* region, expression of *Ubx* becomes progressively lower (Helfand, personal communication). This could be explained by cooperative binding interactions of transcriptional activators to repeated upstream sites. But although the *bxd* region does contain TAA repeats, it is not yet known whether they are required for correct *Ubx* expression, nor is the identity of the proteins binding to the *bxd* region known. Conversely, a recent paper by Weinzierl *et al.*⁹ on mutations in the coding regions of *Ubx* suggests a perhaps

George Khoury (1943–1987)

GEORGE Khoury died on 25 April after several years of battle with lymphoma. He attributed several of his near-miraculous remissions from disease to his own "good protoplasm". He had that and much, much more.

Khoury's reputation as a molecular biologist began in the early 1970s among a small group of workers who pioneered the use of simian virus 40 as a model for eukaryotic transcription and replication. He made the first transcription map of an animal virus, demonstrating the use of restriction enzymes in such mapping studies. This mapping subsequently enabled him to discover that simian virus 40 transcripts are not encoded by contiguous stretches of DNA, an important clue that paved the way for the discovery of RNA splicing. His group at the National Institutes of Health in Bethesda described for the first time the functioning of a eukaryotic *trans*-acting transcriptional regulatory element, the viral T antigen. These results showed that the T antigen acts as a negative regulator of its own messenger RNA synthesis.

More recently, his group showed that the T antigen is a positive regulator of late messenger RNA biogenesis. His work also showed the essential role of splicing in the maturation and export of messenger RNA precursors from the cell nucleus. He

testable hypothesis about the relationship of *Ubx* structure to the function of its products as gene regulatory proteins. Their observation was as follows.

Between the exon encoding the homoeobox at the 3' end of *Ubx* and the major coding portion of the 5' exon lie two so-called microexons, both of which are present in the major *Ubx* transcript (Kornfeld and Hogness) but one or both of which are absent from less abundant messenger RNAs. Weinzierl *et al.* examined the effect of a nonsense mutation in the second of the microexons on the phenotype of the fly embryo. Such a mutation would terminate translation before the homoeobox, and the effect on the epidermis and embryonic nervous system is identical to that of two other mutations destroying the reading frame of all transcripts. During differentiation of the adult central nervous system, however, where *Ubx* is normally also expressed, the mutation has no effect. Although it is possible that this is because adult differentiation of the central nervous system is less sensitive to the loss of the *Ubx* product, the more interesting possibility is that the mutant microexon is normally spliced out of the adult neural transcript so that the mutation has no effect in this tissue.

It would follow from this that the regulatory protein would have subtly different properties in the two tissues; and

pioneered the molecular-biological characterization of the human pavovaviruses BK and JC, and in the early 1980s he and colleagues discovered transcriptional enhancer techniques.

For those who knew George Khoury there were other sides of his character that were as important as his creative, prolific scientific output. He showed by example that even in the heat of intense competition, generosity was preferable to pettiness; that acts of kindness and integrity accrue to one's name far more than do long lists of publications. When others would criticize a colleague, he would invariably muster a more charitable response. He showed that setbacks can be greeted with equanimity. His examples set a standard to be emulated.

In recent years, he became a critical catalyst in the renaissance of molecular biology at Princeton University, his alma mater. The declining energies of his last weeks were spent largely to ensure that colleagues who depended on him would be taken care of. He died three days before the announcement of his election to the US National Academy of Sciences.

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Weinzierl *et al.* provocatively remark that the microexons encode short stretches of amino acids separating the homoeo domain from a second conserved domain and might be expected to affect the functional properties of the protein.

Experiments reported by Mark Ptashne (Harvard University) on *ersatz* looping in bacteriophage λ and the lambdoid phage 434 suggest how. The λ repressor has a flexible stretch of amino acids separating its DNA-binding domain from the carboxyl domain that mediates the protein-protein interactions necessary for dimerization and cooperative binding (see refs 7 and 8), and when its adjacent binding sites are artificially separated will bind cooperatively and cause the intervening DNA to loop out; 434 repressor, which is otherwise similar, has no flexible 'neck' and in analogous experiments fails to induce loops. Further speculation is clearly premature at this stage. □

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Miranda Robertson is Biology Editor of *Nature*.

High-energy physics

Particle production in crystals

Allan H. Sørensen and Erik Uggerhøj

ENERGETIC photons may convert into electron-positron pairs, but only in the presence of an external electromagnetic field — a process called pair production. Often the strong field inside an atom is exploited. Recently, Belkacem *et al.*¹ have demonstrated experimentally that a single crystal provides a very efficient alternative source of field. At a photon energy of 150 GeV, the photo-conversion probability in a germanium sample increases by a factor of about 10, to 0.3 mm^{-1} , if the incident beam is aligned within about 1 mrad of a major crystallographic axis. This effect could be used^{2,3} in novel GeV γ -ray telescopes with high angular resolution, that could be mounted in satellites.

The basic mechanism responsible for conversion of a photon into a particle-antiparticle pair is identical to that enabling a charged particle moving in an external field to emit electromagnetic radiation (Fig. 1). For the latter case, arguments of classical physics suggest that the radiation power is essentially proportional to the square of the product $(E/mc^2) \times Fm^{-1}$ where E is the particle energy, m is the rest-mass, F is the force acting on the particle and c is the speed of light. This implies increased intensities for high energies, strong fields and light particles. With laboratory fields and beam energies available now, the radiation is confined to relatively 'soft' photons, with energy orders of magnitude less than that of the projectile, even using light electrons. For example, synchrotron radiation facilities with GeV particles produce keV photons. Atomic fields, however, are sufficiently strong that a penetrating energetic electron is able to radiate all its kinetic energy into a photon. In pair creation, 'hard' photons are involved as all the primary energy is transferred to the electron and the positron. Hence, pair creation is essentially impossible in laboratory fields whereas it is perfectly possible in atomic fields.

But why should a group of regularly arranged atoms (a single crystal) be introduced as the source of field? The keyword is coherence. Coherent action of crystal atoms is well known from 'channelling' phenomena as we described in a recent review⁴. Consider a charged particle impinging at a small angle on a row of atoms. Such a projectile is subjected to many correlated, soft collisions. The row acts as a whole and the resulting trajectory is essentially identical to the one obtained using a 'continuum' string obtained by smearing the atomic charges uniformly along the row. The strength of the trans-

verse field is of the order of $10^{11} \text{ V cm}^{-1}$.

The coherence in scattering observed at small impact angle also enhances the emitted radiation⁴. The emission of photons by electrons traversing the field of a row of atoms is stronger than if the atoms were distributed randomly. (A classical analogue is constructive interference of radiation emitted at successive scattering centres.) The enhancement obtained is up to two orders of magnitude. For impact energies in the range MeV-GeV, this enhancement is found only for soft photons. In the 100-GeV range, however, the coherent magnification extends to photons with the projectile energy. Conversely, for low impact-angle photons in the 100-GeV range, the probability of photo-conversion into electron-positron pairs is enhanced (Fig. 2a).

The pair-production rates recorded recently by Belkacem *et al.*¹ agree nicely with theoretical predictions. This holds true for the variation with decisive parameters like photon energy and angle to crystal axis. Also the agreement on an absolute scale is quite satisfactory. The discrepancies with theory encountered in previous data⁵ have been eliminated. For photons incident parallel to a crystal axis, the theory developed⁶ for pair creation in strong (homogenous and macroscopic) electromagnetic fields is applicable. Consequently, the experiment can be considered a successful proof both of the significant influence of crystal structure at high energies and of strong-field quantum electrodynamics. The dependence of the effect on target material and temperature,

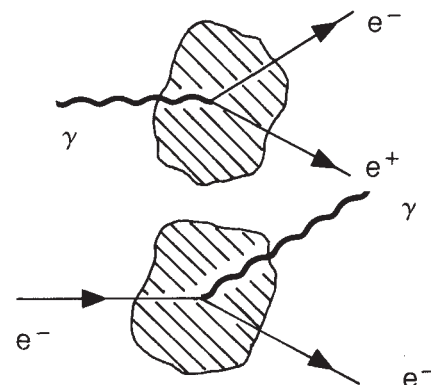


Fig. 1 The equivalent processes, conversion of a photon (γ) into an electron (e^-)-positron (e^+) pair, and photon emission by an electron. Shaded regions symbolize the external field.

and on the energy of one of the produced particles, is yet to be studied.

Along with pair creation, Belkacem *et al.* have also measured photon emission by 150-GeV electrons and positrons incident near an axial direction in a germanium crystal⁷. Strong enhancements are observed for alignments better than 1 mrad. At very small angles, ≤ 0.05 mrad, electrons get focused around the axis whereby they give higher yields and photon energies than do positrons, which are defocused (Fig. 2b). For electron impact, however, the increase in yield observed below 0.05 mrad is gathered in a strong photon peak at 85 per cent of the primary energy. No explanation has been given for this phenomenon. Further experiments on very thin samples are urgently awaited to decide whether a new effect is responsible or if the origin of the peak is trivial, for example caused by two or more photons radiated by a given electron being recorded as one of energy equal to their sum. We have estimated the variation in the total radiated energy at angles ≤ 0.05 mrad (Fig. 2b) and found

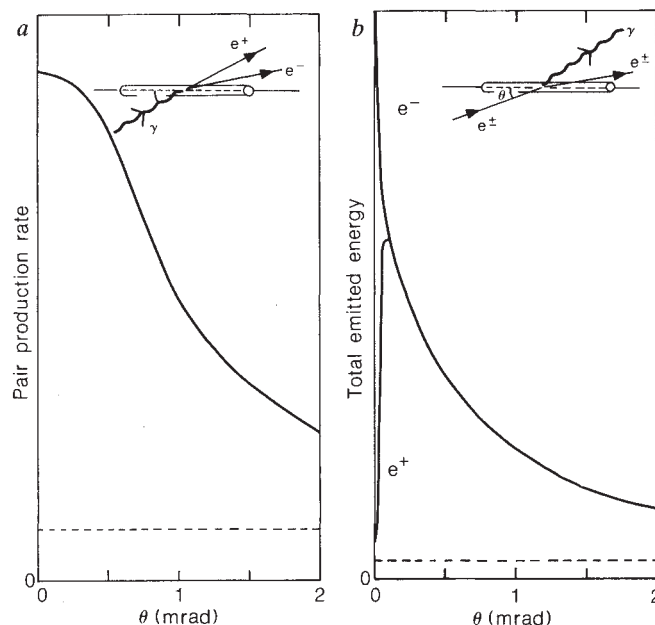


Fig. 2 *a*, Pair-production rate and *b*, photon emission as functions of incidence-angle θ relative to a row of a continuum crystal (inserts) for impact in the 100-GeV region. Dashed lines indicate the level obtained far from axes (and planes). The coherent action of the crystal, as manifested through the macroscopic continuum rows, leads to strong enhancement at small angles.

good agreement with the observations.

Recently, our own group has performed experiments at CERN similar to those reported in refs 1 and 7. For pair creation, the results confirm those of Belkacem *et al.*¹. For radiation, preliminary analysis of data for electrons penetrating a very thin silicon crystal reveals a further increase in the yield for impact closer than 0.05 mrad to the axis, as reported in ref. 7. Unfortunately, it is not possible to draw a conclusion on the occurrence of a hard-photon peak because of poor counting statistics.

Finally, are there any applications? We have mentioned above the possibility of direction-sensitive, hard-photon detectors^{2,3}. An energetic (≥ 100 -GeV) photon has a high probability of pair-production in a 2–3-mm-thick germanium crystal, provided it enters within about 1 mrad to a major axis. This feature could be exploited to improve, by several orders of magnitude, the angular resolution in locating hard-photon sources in the Universe. A very simple piece of equipment is obtained if the converting crystal itself is a solid-state detector measuring the conversion rate. Also, coherence in crystals

could enhance the intensity of particle beams produced in high-energy laboratories. Both applications depend on the development of a cascade — a succession of the processes shown in Fig. 1, by which a single, high-energy photon converts into many low-energy photons, electrons and positrons. At high impact energies, cascades develop fully in approximately 1 cm of aligned germanium, whereas 10–50 times more non-aligned material is needed: tests with thick aligned samples were performed some years ago⁸. The use of tungsten rather than germanium would extend the useful range to lower photon energies. □

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Plate tectonics

Drifting mantle hotspots

Peter Olson

PLATE-TECTONIC motions have erased nearly every coordinate system capable of determining the absolute location on the Earth's surface of past geological events. Two terrestrial reference frames not obviously affected by seafloor spreading are the main geomagnetic field, whose time-averaged state is nearly a geocentric, axial dipole; and the mantle hotspots, long-lived centres of volcanic activity thought to be fixed with respect to each other. Now, even these reference frames, particularly the hotspots, appear to be mobile, according to results presented by Sager and Bleil¹, and by Molnar and Stock on page 587 of this issue².

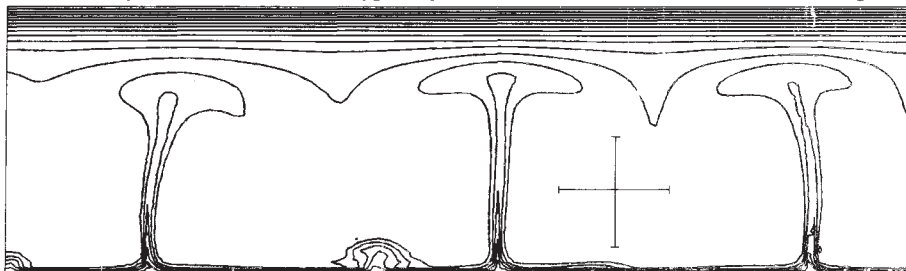
J.T. Wilson³ was the first to recognize that certain chains of volcanic islands such as Hawaii show a progressive increase in age in the direction of plate motion and seem to be tracks originating from localized hotspots fixed in the mantle beneath the plate. W.J. Morgan⁴ established their importance in geodynamics, proposing that hotspots are stationary with respect to each other and define an irregular, rigid reference frame; and that they result from thermal plumes rising from deep in the mantle (see figure). The first point — the fixity hypothesis — can be tested by reconstructions of past plate movements. Early studies, based either on global datasets for the past 10 million years (Myr) or older data from slow-moving plates, generally

supported the concept of fixity, showing little or no deviation between plate trajectories and hotspot tracks. This indicated that the source of hotspots, the deep mantle, must remain virtually undeformed while the lithospheric plates and the upper mantle circulate above it. But this rather simple picture conflicts with recent results from seismic tomography that show large-scale lateral heterogeneity in the lower mantle⁵ and undulations of ± 6 km in the core–mantle boundary⁶, both strong indicators of vigorous convection in the lower mantle. Large-scale convection in the source region should cause plumes to drift and make hotspots move with respect to both the plate above and the hotspots under adjacent plates. The contradiction would be resolved if hotspots do move about in the mantle, but more slowly than the sea floor typically

spreads. On kinematic grounds the apparent drift of a hotspot produced by a deep plume should be sensitive to the net sublithospheric velocity in the vertical cross-section of the mantle through which the plume ascends, which would be small compared with the plate velocity and might be nearly in the opposite direction.

To resolve small relative motion (millimetres per year), old hotspot tracks on fast-moving plates must be used, and that is the approach of Sager and Bleil, and of Molnar and Stock, using essentially independent methods. Sager and Bleil use palaeolatitude determinations derived from Deep Sea Drilling Project cores from the western Pacific to show that the geomagnetic latitude of the Hawaiian hotspot has decreased at a rate of about $0.3^\circ \text{ Myr}^{-1}$ between 70 and 40 Myr ago. During this time the Pacific plate moved north and the Hawaiian hotspot was producing the Emperor Sea Mounts. As palaeomagnetic data of this type do not resolve changes in longitude, only changes in hotspot latitude can be determined using Sager and Bleil's approach. Also, because their comparison is based on the position of a single hotspot relative to the geomagnetic pole, some ambiguity enters when interpreting their results. For example, the relative motion could partly result from drift of the geomagnetic dipole, rather than just the hotspot. Theoretical arguments, supported by extensive palaeomagnetic data from the recent past, strongly indicate that the main dipole field tends to align along the Earth's axis of rotation, so that motion of the geomagnetic pole is tantamount to motion of the axis relative to surface features. This is called true polar wander (TPW) and there is some evidence that this has occurred to a few degrees of latitude over the past 40 Myr. Sager and Bleil show that TPW, with the spin axis moving away from the Pacific Basin, can explain their results.

But from a geodynamical point of view, a more likely explanation is that the hotspots are moving. A direct test of this alternative has been made by Molnar and Stock, without making any direct appeal to the positions of the palaeomagnetic pole. These authors show results of a detailed comparison between the tracks of Hawaii and hotspots in other oceans with their predicted tracks, calculated using the



Temperature contours from a numerical simulation of thermal plume formation from the mantle D'' thermal-boundary layer and hotspot formation by interaction with the lithosphere. The scale bars are 200 km (horizontal) and 100 km (vertical). (Courtesy of G. Schubert and C. Anderson.)

fixity assumption. The calculations require a fairly precise model of relative plate motions during the past 65 Myr. Even including errors in plate motions, Molnar and Stock find systematic displacements between Hawaii and the other hotspots (Iceland and Tristan de Cunha in the Atlantic; Reunion, St Paul's and Kerguelen in the Indian Ocean). The inferred relative velocities are generally small — typically an order of magnitude less than the Pacific plate speed of 10 cm yr⁻¹ — but they do indicate a southwards drift of the Hawaiian hotspot, consistent with the results of Sager and Bleil.

Although these warps in the hotspot reference frame are a nuisance for those who are trying to reconstruct precisely past plate-tectonic configurations, they seem to offer a solution to one of the more enigmatic problems in mantle dynamics, namely, how do hotspot sources remain fixed in the presence of large-scale convection? The most likely physical mechanism for producing hotspots is by thermal plumes originating as instabilities in a hot

low-viscosity boundary layer at the base of the mantle, the seismically defined D''-layer⁷, as shown in the figure. A difficulty with this model arises if large-scale convective motions, driven primarily by subducted slabs, are superimposed. The sites of plume formation shown in the figure will travel with the large-scale circulation and the hotspots will appear to drift, perhaps generally counter to surface-plate motion and, in any event, with respect to each other. The relative southwards drift of the Hawaiian hotspot at the time when seafloor spreading in the Pacific was northwards seems to confirm what is expected from the plume model. □

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Climatology

Plant growth and CO₂ history

J. I. L. Morison

EARTH'S atmospheric carbon dioxide concentration is increasing at a well-publicised rate of around 1.5 parts per million (p.p.m.) per annum from the present-day level of 348 p.p.m. Much effort is being spent on predicting the changes that this will cause to global climate and the effect that this increase will have on plant growth. On page 617 of this issue¹, however, Woodward reports the startling result that the leaves of several tree species collected recently from central England differ anatomically from herbarium specimens collected over the past two centuries: the number of pores per area of leaf has declined steadily by about 40 per cent during this time. This result raises important points for environmentalists, biologists and palaeoclimatologists to consider.

The pores (stomata) in leaves maintain a suitable balance between CO₂, necessary for photosynthesis and growth, and the unavoidable loss of water from the wet interior of the leaf. The cells surrounding the pores respond to light, humidity, temperature and CO₂, apparently to optimize the rate of exchange between the CO₂ gained and the water lost. Many experiments have shown that the rate of exchange (the water-use efficiency) can be increased by increasing the CO₂, which could have profound implications for plant growth in past or future atmospheres. The number of stomata per unit leaf area (stomatal density) varies between leaves of different species and

between leaves of similar plants grown in different conditions. It is surprising that Woodward has been able to distinguish a clear decrease in stomatal density by examining herbarium specimens that may well have been growing in widely varying conditions before collection. Woodward concludes that the decrease is caused by the increase of 60 p.p.m. CO₂ over the past 200 years, because plants experimentally grown in low CO₂ concentrations show increased stomatal density. Not only that, but the plants grown at the pre-industrial concentration show water-use efficiencies less than half that of plants in today's atmosphere.

These results are novel because the wealth of previous experimental work has generally been directed towards plant growth in future atmospheres (with higher CO₂ concentration), where little change of stomatal density is predicted. Together with previous studies, this emphasizes that the global rise in CO₂ is already having important effects on the biosphere. A comparable effect to that seen by Woodward has been observed in tree-ring studies at the University of Arizona², where Fritts and colleagues can detect an increase in the growth rate of bristlecone pines across the western United States. This is related to the increase in CO₂ because the reduced pressure at the high altitude where these trees grow makes photosynthesis particularly sensitive to small increases in CO₂ as a fraction of the

air mixture. Similarly, it has been suggested that the increased amplitude of the seasonal variation of atmospheric CO₂ concentrations measured at Mauna Loa and other stations around the world³ is a sign that the biosphere is responding to the increase in CO₂. These observations are important because most climatologists agree that it may be several years, even decades, before the effect of CO₂ on the climate can be distinguished from the normal variability.

Climatologists are intrigued by the possibility of using information on climate patterns in earlier, warmer epochs as historical analogies of what we should expect in a warmer, high-CO₂ world. Indeed, it is known that some of the temperature fluctuations over the past 100,000 years are linked to changes in atmospheric CO₂, although the question of a causal relationship is still debated. For example, concentrations of 200 p.p.m. are typical in glacial periods and are 40–80 p.p.m. lower than during interglacial periods⁴. Woodward's results suggest that where leaf material available, changes in stomatal density could be found over these prolonged periods. Plant species presumably grew in the same local climatic conditions, although these varied geographically between epochs. But if the variation in CO₂ caused significant variations in the water-use efficiency, then species distribution would be affected and the argument becomes circular. Indeed, some of the palaeoclimatological evidence for climate change does come from information on plant species distributions.

Is it really likely that there has been a substantial change in water-use efficiency over the past two centuries? Although such an effect of increased CO₂ can be readily demonstrated on a small scale in glasshouses and field enclosures, there are several physical reasons for believing that the effect is much reduced outdoors and on a larger scale: first, the balance between energy supply and water loss from leaves; second, the interaction between evaporation from vegetation and the humidity above that vegetation and over the whole region; and third, the compensatory feedback between evaporation from leaves and that from the soil underneath^{5,6}. The answer should reveal how plant growth over the whole land surface will respond to future increases in CO₂. □

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Schrödinger's entropy and living organisms

SIR—Perutz (*Nature* **326**, 555–558; 1987) may not have done justice to the argument of Boltzmann and Schrödinger about negative entropy and living organisms. Consider an isolated system that contains a living organism. The organism is an open system, exchanging mass-energy with its surroundings. It maintains itself in a stationary state of approximately constant entropy. Since the total entropy of the isolated system, which is not at equilibrium, must increase with time, the living organism must continually transfer positive entropy to its environment, or, expressed in other words, it must be a sink for negative entropy. A picturesque way to express this flux is to say that the organism feeds on negative entropy.

Where Schrödinger was misleading was in interpreting this feeding too literally by emphasizing the ordered structures of nutrients as the source of negative entropy. Actually the net negative entropy flux is the sum of all the entropy fluxes across the boundary of the organism, and the metabolism of individual nutrients may contribute positive or negative terms.

The other thermodynamic considerations cited by Perutz are irrelevant to the argument in "What is Life?"

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Jason's golden fleece explained?

SIR—Many explanations have been offered for why the fleece sought by Jason and the Argonauts should have been described as 'golden'. For example, Strabo¹ suggested that the fleece was used to trap gold washed down the river Phasis. More recently it was suggested that the term golden may have referred to the fineness, and the related value, of the fibre².

I should like to suggest instead that the fleece was literally a golden-yellow colour. This type of wool discolouration is observed in sheep which have sustained liver damage that prevents normal excretion of bilirubin in the bile. Instead, bilirubin and some related pigments appear at the skin and in the suint, resulting in a yellow staining of the wool^{3,4}. This condition is frequently the result of ingestion of hepatotoxins which are present in some plants. Many of these hepatotoxins, identified notably in *Lantana* species, are members of a group of related pentacyclic triterpene acids⁵. Although the parent triterpene, oleanolic acid, does not display icterogenic activity in rabbits at the levels studied⁶, in recent work it was found to be a potent toxin for cultured rat hepatocytes

when administered at concentrations above 'threshold' value⁷.

A central feature of the golden fleece legend is that of famine. According to the story related by Apollodorus⁷, the famine was contrived by a jealous stepmother in order to rid herself of her stepchildren. She persuaded her husband that the only way to end the famine was to sacrifice the children. However, they were rescued by a ram with a golden fleece. In times of drought in New Zealand, when no other feed remained, farmers have resorted to feeding their sheep on leaves from trees. Perhaps during the famine recalled in the golden fleece story, sheep would similarly have been fed on leaves from the olive tree, which was extensively cultivated in Mycenaean Greece. Olive leaves are particularly rich in oleanolic acid: it has been estimated that oleanolic acid accounts for at least three per cent of the total fresh leaf weight of *Olea europaea*⁸. If a sheep is fed principally on olive leaves and, more specifically, leaves from trees that have experienced drought stress, the sheep might acquire a dose of oleanolic acid (or a related triterpenoid) sufficient to produce the liver damage that gives rise to a golden-yellow discolouration of the fleece.

Although this explanation of the golden fleece is speculative, it is at least amenable to an experimental test.

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Priority claims policy and disenchantment

SIR—In a recent editorial¹ you commented on the undesirability of authors describing their work as "first"; and the managing editor of the *Proceedings of the National Academy of Sciences U.S.A.* subsequently stated² that the policy of that journal is not to print priority statements. The desirability of such a policy is demonstrated by a paper in the same issue of *Nature* by Dandanell *et al.*³, which was submitted on 8 September and accepted on 4 December 1986.

The authors state "Here we report that transcription initiation can be regulated from an operator site placed 1 to 5 kilobases (kb) downstream of the *deoP2* promoter (and downstream from the transcribed gene), and present the first

experimental data for prokaryotic regulation at distances >1 kb". But they do not refer to our paper which appeared in *Cell* of 20 June 1986⁴ and in which it was shown that transcription of *glnA* is stimulated by the presence of binding sites for the regulatory protein located 1.4 kb upstream or 2 kb downstream from the *glnA* promoter.

Moreover, Dandanell *et al.* consider the "far" site in the *deo* system to be analogous to the enhancers of eukaryotic cells. However, in the eukaryotic systems and in the prokaryotic *glnA* system, binding of the regulatory protein to sites far from the promoter activates the initiation of transcription, while in the *deo* system, binding of the regulatory protein to a site far from the promoter blocks the initiation of transcription. The analogy in this case seems to be to as yet undiscovered eukaryotic disenchanters.

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DANDANELL *et al.* reply — In three articles^{1–3}, we have analysed gene regulation in *Escherichia coli* in a system under the control of a repressor that binds to multiple sites on the DNA that are separated by considerable distances, and we have discussed the striking similarity between upstream and downstream control regions in bacteria and that of enhancer elements in eukaryotic genes. With reference to the last article, we apologize for not referring to the work of Reitzer and Magasanik⁴ and agree that it is undesirable for authors to describe their work as "first".

We do not, however, agree with Magasanik's reflections on enhancers/disenchancers. First, the central issue is not positive versus negative control, but how proteins, present in a very low concentrations in the cell, are able to 'find' their specific targets on the enormous DNA molecule and occupy these sites efficiently. One way to overcome the competition between nonspecific and specific sites for a DNA-binding protein is DNA-looping which involves cooperative binding to two or more targets. This model is general and not restricted to positively acting proteins. Second, it is clear that a regulatory protein can act both as an activator and a repressor or that an effector can change a repressor to an activator (as in the classical prokaryotic systems, bacteriophage λ and the *ara* operon). Present evidence suggests that similar phenomena also exist in eukaryotes. Thus, the E1A early antigen of human adenovirus, known to activate transcription from many promoters, is also able to function as a repressor (see ref. 5). Third, both negative and positive

control systems coexist not only in bacteria but also in eukaryotes. Recent work has provided a spate of information about negative regulation of eukaryotic genes, and it seems likely that repressor-like molecules play an important role in differentiation⁶. Furthermore, DNA sequences, called silencers, with properties opposite to those of enhancers, have been described^{7,8}. Finally, we would like to stress that the interplay between regulatory proteins and multiple DNA targets through DNA-loop formation provides a very flexible framework for control and may well prove to be the clue for an understanding of other regulatory phenomena.

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Bamboo shortage not only cause of panda decline

SIR—O'Brien and Knight in their article "The future of the giant panda" (*Nature* **325**, 758–9; 1987) make a misleading statement which requires comment: "As a result of the flowering of the arrow bamboo in Wolong in 1983–85, 40–50 per cent of its pandas may have died and others may have emigrated".

There has been no mass starvation in Wolong. Neither the field biologists on the panda project nor the Chinese monitoring teams (who live in remote parts to find and assist debilitated pandas) have found evidence for such an event. In fact, field surveys—including my own—in various parts of Wolong revealed that patches of arrow bamboo survive throughout the area and also that pandas have alternative bamboo species available as food. In the project's main study area, pandas actually continued to subsist on arrow bamboo for three years after the die-off, showing food selectivity and activity patterns as before, until the winter of 1986–87 when some animals began to increase their use of the low-elevation umbrella bamboo. No evidence for emigration from the reserve exists; in any

case, habitat conditions in neighbouring areas are no better than in Wolong.

The 1986 census of Wolong pandas revealed 72 animals, about half as many as a decade earlier. Whatever the precise figures, there has been a decline. What is the cause? During 1982–83, two and most probably three pandas in our small study population are known to have died in snares set by poachers for muskdeer. Such snaring was common in Wolong as well as in many other panda habitats both inside and outside reserves. Slow, constant attrition through snaring has no doubt been a major cause of the decline of Wolong's pandas and of those in various other areas.

Outside Wolong, a number of pandas starved as a result of the bamboo die-off in several small localities. China's captive population has increased greatly in recent years, with most pandas that were rescued during the die-off or otherwise captured having not yet been rehabilitated to the wild — as they should be, given the panda's poor breeding record in captivity. Taken together, the various factors can explain the overall decline in the number of pandas during the past decade. On completion of the current census in 1988, a reliable figure on the current status of the species will become available.

Conservation efforts must be based on accurate information and a recognition of priorities. Pandas are not timid and they readily adapt to the vicinity of humans. But they do need ample bamboo in relatively undisturbed forest habitat. And being a slowly reproducing species, the animals cannot maintain themselves under heavy hunting pressure. To preserve habitat, control poaching, and return many captives to the wild are three urgent conservation priorities.

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Insects and Cretaceous mass extinction

SIR—In the continuing debate¹ on the reality and cause of the mass extinctions at the end of the Cretaceous, little attention has been paid to insect faunas. I should like to point out why comments on the "almost complete absence in the Cretaceous"² of insect fossils are ill-founded, and how insect fossils can contribute to this debate.

Based on the morphology of living insects, most of the orders of insects and many of the families have been recognized in the Cretaceous. These include, for example, Formicidae (Hymenoptera), Aeshnidae (Odonata), Micropterigidae (Lepidoptera), Curculionidae (Coleoptera), Aphididae, Anthocoridae, (Hemiptera), Ceratopogonidae, Stratiomyidae (Diptera). These families, amongst many

others known from the Cretaceous, have species which are common and widespread today. Most are well represented in the Cretaceous fossil record^{3,5} suggesting a widespread and abundant fauna. Across the Cretaceous/Tertiary (K/T) boundary the morphology of the individuals, and to a large extent the faunal composition, have changed little.

With their rapid reproductive rate and great powers of recovery, even over tens of years, is it possible that insects recovered so rapidly from a major catastrophe that changes in faunal composition at the K/T boundary are so far undetected? What effect would the proposed meteorite impact⁶ have on insects?

Based on extant insect populations and on dividing the world insect fauna into three divisions — temperate northern, temperate southern and tropical latitudes — my answer is as follows. During the northern winter insects in the north will be in diapause, while Southern Hemisphere insects and those in the tropics will be active. Conversely, northern zone insects will be active during the southern winter. If a global period of cold and darkness following a meteorite impact lasted only a few months, insects in the winter latitudes could regard it as a 'late Spring' and remain in diapause for the extra months. In tropical forests the insect fauna, which does not have a comparable rest period, would be seriously affected, as would the insects in the summer latitudes. If the temperature and day lengths in the summer latitude were only gradually reduced after a meteorite impact, then insects in such regions might be able to respond by going into diapause. But even under these conditions the tropical insect fauna would be seriously depleted.

Assuming survival of insects in northern or southern temperate latitudes after a meteorite strike, the tropics would have to be recolonized by insects that survived in a temperate zone, a hypothesis for which there is no current evidence.

There is need for a closer study of the insect fauna across the K/T boundary. At present the evidence points to survival of families, with apparently lower survival rate at the generic level and little or none at the species level. Lack of evolutionary change in the insects across the K/T boundary argues against abrupt or catastrophic changes.

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The design of disaster

Alvin M. Weinberg

Chernobyl & Nuclear Power in the USSR. By David R. Marples. *Macmillan, London/St Martin's Press, New York:1987. Pp.228. Hbk £25, \$35; pbk £8.95, \$14.95.*

CHERNOBYL, according to David Marples, "has demonstrated that the Soviet nuclear industry has been operating in a manner that bordered on the foolhardy. And at present, there have been few indications that Chernobyl will change anything or that it will be the last such nuclear disaster." To his rhetorical question "Does the Soviet industry constitute a living danger for the world at large?", the author all but answers yes.

Marples is an economist at the Canadian Institute of Ukrainian Studies. *Chernobyl & Nuclear Power in the USSR* is a summary of Soviet and Eastern European, as well as a few Western, newspaper accounts of the accident and its broader setting. Because his sources are hardly available to most Westerners, Marples gives us an unusual perspective on the accident itself, as well as an impression of how Eastern commentators have viewed their own nuclear enterprises.

Unfortunately, the author is not an expert in nuclear technology, and his technical exposition often lacks verisimilitude. More importantly, despite his claim of political neutrality, Marples rarely passes an opportunity to draw inferences that are almost always unfavourable to the Soviets. His conclusion as to the future threat posed by Soviet reactors must therefore be examined most carefully, especially because its implication is so devastating.

Marples adds little to what is generally known in the West about the Chernobyl accident itself, especially because most of the book seems to have been written before the Soviets presented their account of it at the International Atomic Energy Agency (IAEA). This official version is relegated to a brief epilogue. Marples's lack of technical expertise is most evident in his handling of the accident. Thus he all but ignores the main difficulty at Chernobyl — the positive void coefficient of the RBMK reactors, which makes such reactors prone to a nuclear runaway should they be deprived of cooling water. What Marples does add are fascinating journalistic vignettes and human interest stories about the evacuations with their apprehensions and confusions. Many of his sources are Ukrainian; he notes that Cher-

nobyl is only one of many misfortunes visited upon the Ukraine since the revolution. For Marples, the accident at Chernobyl carries important ethnic overtones that are lost on neither the Ukrainian nor the Soviet Communist Party.

Marples is at his best when he describes the trials, tribulations and, occasionally, the successes of nuclear energy in the Ukraine and in the Eastern Bloc. The Soviets have traditionally been less than forthcoming in describing progress at their

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Explosive consequences — the ruins of Chernobyl's No. 4 reactor.

huge, multi-reactor stations such as Chernobyl or Zaporizhzhia — the latter a nuclear park scheduled to contain seven 1,000-megawatt RBMK reactors. Marples documents many instances of poor workmanship, slipped schedules and cost overruns. Yet in all fairness, completing the first two 1,000-megawatt reactors at Zaporizhzhia in less than eight years is not a bad record; and some reactors in the United States and Britain have also suffered from poor workmanship, slipped schedules and cost overruns.

The relation between the Soviet nuclear programme and that of the rest of the Eastern Bloc is described in some detail. There are no RBMKs outside the Soviet Union (except for the roughly similar Hanford N-reactor, which is also water-cooled and graphite-moderated): the Soviets have exported only VVER pressurized water reactors, mainly to the Eastern Bloc. And although Marples leaves the impression of shoddiness and deficiency in

these systems, this is not the view of Western nuclear technologists. For example, the Paks VVER station in Hungary, and the Loviisa VVER station in Finland have both been operating remarkably well for years. (Perhaps more important, the young chief engineer at Paks struck me, during a visit there last year, as being equal, and in some ways superior, to chief engineers at US nuclear plants.)

Marples attributes the deficiencies in the Soviet nuclear enterprise to the decision, taken many years ago by the Soviet government, to depend on nuclear electricity to displace coal in the western Soviet Union. The ensuing emphasis on speed and on paring costs has led to poor quality assurance, shoddy construction and cutting of corners on safety. The decision itself was a defensible one, in view of the

enormous burden on the railroad system that would have been imposed had fission not been exploited; but insofar as safety was subordinated to speed or economics, the consequences have been distressing.

Do these shortcomings justify the conclusion that the Soviet nuclear enterprise is a living danger for the world at large? To support such an assertion one must demonstrate that, whatever their past transgressions, the Soviets are unlikely to do better in the future. Two technical points are of significance here: the use of containments and the design of future RBMK reactors. Western nuclear technologists had always been sceptical of the Soviet claim that full containments were not needed for their reactors — a practice

that was undoubtedly prompted by economic considerations. The incident at Three Mile Island proved that full containments were essential; and Soviet pressurized water reactors (PWRs) built since then are contained. On the other hand, full containment of future RBMK reactors is unlikely and containment of existing RBMKs is impossible. Secondly, once the Soviets realized that the accident at Chernobyl would not have happened had the RBMK reactor's void coefficient been negative rather than positive, they announced plans to increase the enrichment of the RBMK fuel so as to achieve a negative void coefficient as well as incorporating various other improvements. Thus, assuming these and other changes are put into practice, the Soviet RBMK reactors will be considerably safer than they have been. Whether they will then be as safe as fully contained Western reactors, or for that matter Soviet PWRs, is arguable. ▶

The Soviet penchant for secrecy, as well as its rigidly hierarchical structure, undoubtedly contributed to the ordeal at Chernobyl. Western reactors and reactor designs are criticized, especially in the United States, *ad nauseam*, by independent bodies such as the Nuclear Regulatory Commission as well as by pressure groups. Though public hearings and the like are costly and often even self-defeating, on balance I would say reactors in the United States today are probably safer than they would have been without them. Critics of the nuclear industry in the Soviet Union were largely unheard of until Chernobyl. But beyond this, the reluctance of the Soviets to share operating details with the rest of the world has deprived their system of international peer criticism, though since Chernobyl they have endorsed the idea of sharing, through the IAEA, their experiences with the rest of the nuclear community.

Was Chernobyl the inevitable consequence of a top-heavy and isolated bureaucracy's insensitivity to safety in favour of keeping costs down, as Marples claims; or was it the result of an incredible sequence of operating errors and generally poor performance by those in charge of the reactor, as the Soviets claim in their report to the IAEA? Certainly, both of these factors were involved, but in my opinion they are secondary. The original RBMK reactor design was flawed in having a positive void coefficient: the designers of RBMK must therefore share the blame for the accident with the operators. Could the designers have been expected to remedy this deficiency before RBMK was built? The designers of the Hanford N-reactor eliminated the positive void coefficient even though this added a little to the cost of operating the reactor. Perhaps the RBMK designers were under more pressure to put economics over safety than were their American counterparts — but this is hardly provable.

Despite his political bias, Marples has produced an account of Soviet nuclear matters that most of us in the West are unfamiliar with. But I believe he is too hasty in his judgement that the Soviet nuclear enterprise is a danger for the world. At the IAEA, the Soviets promised to implement many improvements in reactor design, safety procedures and management. Should they carry out these reforms, then Soviet reactors would be adequately safe. Nevertheless, given the previous policy of secrecy, the world will remain properly sceptical until Gorbachev's *glasnost* penetrates the Soviet nuclear power industry. All reactors, in the West as well as in the East, will be safer for it. □

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Before the deluge

H.S. Smith

Temples and Tombs of Ancient Nubia: The International Rescue Campaign at Abu Simbel, Philae and Other Sites. Edited by Torgny Säve-Söderbergh. UNESCO/Thames & Hudson: 1987. Pp.256. £25, \$29.95.

THE construction of the High Dam above the First Cataract of the Nile to provide perennial irrigation, an increase in cultivable land and hydroelectric power for an overpopulated Egypt was decided upon in 1954, and effected between 1960 and 1970. It created Lake Nasser, which stretches 500 km southwards to the Dal Cataract in the Sudan, with an average width of 10 km and a capacity of $1.57 \times 10^8 \text{ m}^3$ at 180 m above sea level.

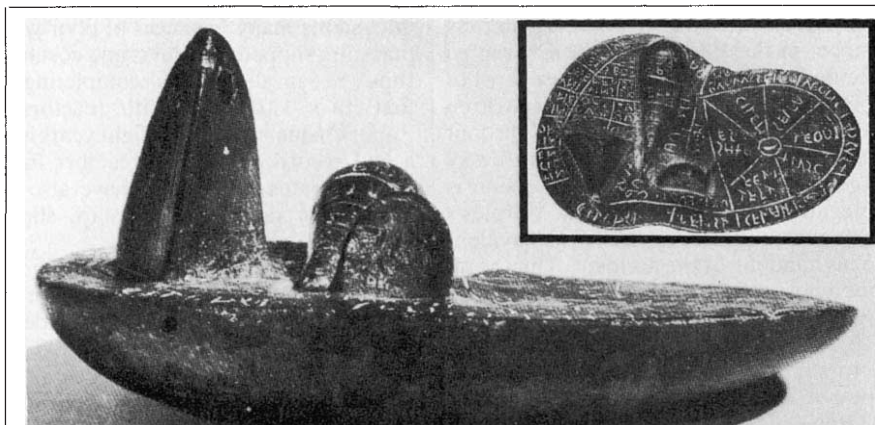
The lake submerged the historic land of Nubia, and caused the deportation of the Egyptian and Sudanese Nubians against their will. To the technical and political problems was added the cultural problem of rescuing the splendid sites and monuments of prehistoric, Pharaonic, Christian and Muslim Nubia. After much deliberation, in 1959 Egypt and the Sudan appealed to UNESCO for international help. The campaign then launched succeeded in many of its aims — in raising the great rock-cut temples of Ramesses II at Abu Simbel; in re-siting the lovely temples of Philae on a higher island in the First Cataract; in removing all other Pharaonic temples and tombs of particular interest to safer locations; in investigating archaeologically most of the sites of Nubia; in discovering and restoring the marvellous Christian frescoes of Faras; and in rescuing the multi-lingual documents of the fortress of Qasr Ibrim. This book is a history of the campaign. It was commissioned by UNESCO, and is of universal interest as a record of the greatest

international cultural collaboration of modern times.

As the pioneer historian of Nubia and a member of the executive committee of the campaign, Professor Torgny Säve-Söderbergh was an ideal choice to edit the book. Though he has clearly consulted a wide range of UNESCO and national records, and quotes contributions from colleagues with due acknowledgement, he has written the work himself in lucid, well-modulated English prose. The book contains a summary of Nubian history and geography, a complete account of the background to and organization of the campaign, a description of the monuments and of their removal, and an analysis of the archaeological surveys and excavations. Säve-Söderbergh has accomplished admirably a difficult task of compression, and the work is beautifully illustrated and produced.

The fascination of Nubian history is that the land, though arid, was of vital importance to Egypt both as a trade route for African products (ivory, ebony, spiced woods, incense and exotic fauna) and for its native wealth in gold, cattle, mercenaries and slaves. Egypt played a major role in Near Eastern politics and culture when it controlled Nubia; when it did not, native Nubian cultures burgeoned. This story is told clearly and succinctly, indeed perhaps too succinctly: uncharacteristic minor errors (for example, on page 35, Sesostri III for Sesostri I, and Buhen for Semna) suggest last-minute cutting.

The account of the launching and organization of the campaign is clear, well documented and on the whole assesses fairly the contributions of Egypt, the Sudan, UNESCO, other nations, and the host of individual donors, scholars, administrators, engineers and media people from all over the world. UNESCO initially concentrated upon salvaging standing monuments rather than archaeological survey and excavation. In Sudanese Nubia, where there had been no prev-



Heaven on Earth? The Bronze Liver of Piacenza, a religious relic of the third century BC; the markings on the upper surface (inset) are interpreted by some scholars as a cosmological map. The pictures are reproduced from Vol. 1 of *The History of Cartography*, edited by J.B. Harley and David Woodward, and recently published by University of Chicago Press.

ious surveys, this second task was vital, and just tribute is given to the Sudanese Antiquities Service and their UNESCO adviser, Professor W.Y. Adams, for their organization of the archaeological work.

In Egyptian Nubia, though late sites like Qasr Ibrim and Gebel Adda were of great importance, the task of raising and removing temples was paramount. Abu Simbel, Philae, Kalabshah and Amada receive the fullest treatment, though other salvage operations are accorded their due. The technological and financial problems, and the reasons for the compromise solutions finally adopted, are presented with clarity, judgement and understanding. This section will be a textbook for future collaborative cultural endeavours. Säv-Söderbergh emphasizes the equal roles of UNESCO's guidance, of international generosity, and of the collaboration of the Sudan and Egypt; Egypt alone contributed nearly half the cost of rescuing the Abu Simbel and Philae temples — a

fact that is not always appreciated.

The account of the archaeological excavations and surveys is again too brief to do complete justice to the variety of the work achieved and the original contributions to scientific knowledge, but several of the most telling examples are described. Some mistakes were undoubtedly made. But the history and archaeology of Nubia were substantially rescued in the limited time available, and the final verdict that the campaign was an "outstanding success" is justified. Those of us who took part will never forget the spirit of camaraderie that prevailed, despite all the difficulties, nor the brave people of Nubia, to whom the final accolade is deservedly given; may future technical triumphs restore them to their beloved land. □

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Physics on a plate

J.H. Mulvey

The Quantum Universe. By Tony Hey and Patrick Walters. *Cambridge University Press*: 1987. Pp.180. Hbk £27.50, \$44.50; pbk £9.95, \$16.95.

RICHARD Feynman, the guru pictured at the front of *The Quantum Universe* and quoted at the start of every chapter, has explained how his formulation of quantum electrodynamics, using the diagrams carrying his name, was the outcome of attempts to understand the motions of a spinning plate. As he told his boss at the time, Hans Bethe, this was an exercise "of no importance whatever, I'm just doing it for the fun of it".

Tony Hey and Patrick Walters believe they should communicate the enjoyment and fascination they find in quantum mechanics to a wider circle of people interested in science by way of a book for their coffee tables. But they also have a more serious intent: to reach those such as the research director of a high-technology firm who has dismissed quantum mechanics as an irrelevancy, so betraying a failure of comprehension which must raise doubts about the future of his enterprise. No reader can fail to be impressed by the way quantum mechanics, replacing newtonian mechanics in the control of particle behaviour at the atomic scale and below, underlies our understanding of the physical world: chemistry, the solid state, microelectronics, lasers, superconductivity, stars and black holes. It is the framework on which physicists construct theories of the forces which have determined the evolution of the Universe from

the Big Bang to the present.

A violin string vibrates in a discrete set of modes. The wave-like formulation of quantum mechanics is used to explain the similarly quantized energy levels of electrons in atoms. But this success is bought at the price of a loss of precision in describing the motion of individual particles. This troublesome counter-intuitive behaviour, to which Einstein was never reconciled, is discussed in a clear analysis of the double-slit experiment. As Feynman said "... nobody understands quantum mechanics"; but, like the physicists, one has to accept that it works and this the authors marvellously demonstrate, commendably resisting any temptation to indulge in philosophical digressions. Simple mathematical relationships are used sparingly, when they really help to convey a point (the more mathematically inclined are invited to solve Schrödinger's equation in an appendix). The text is liberally supported by excellent pictures and diagrams, and scattered with thumbnail biographies of many leading scientists.

By tackling concepts that might have been considered too difficult for non-specialists the authors have boldly broken new ground. The pace never slackens and towards the end the double-slit experiment is again used to illustrate the meaning of gauge (or phase) invariance, the principle which seems to be the key to understanding nature's forces. This book will amaze, baffle and delight as it opens up a view of the physicist's secret garden for those happy to make some intellectual effort to appreciate how the world is as it is. □

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Trust in medicine

Joseph A. Cook

Physic and Philanthropy: A History of the Wellcome Trust 1936–1986. By A. Rupert Hall and B.A. Bembridge. Cambridge University Press: 1986. Pp.479. £25, \$39.50.

In 1880, Henry Wellcome and Silas Burroughs formed a partnership to introduce the American practice of wholesale manufacturing of ready-made compressed pills, called "Tabloids", into Britain. The company's early success was due largely to showmanship and sales of "non-ethical" products such as cod liver oil with malt extract. However, Henry Wellcome, the sole owner after Burroughs's death in 1895, developed great respect for medical research and sought to exploit the opportunities it offered for his business. As the puritanical son of a missionary, Wellcome was also ever mindful of a wish, expressed at age 21, "to live a life devoted to the true God and to mankind". Combining this desire with that of ensuring the continued success of his company, on his death in 1936 he left the entire business to his Trust; profits were to be invested in medical research for the public benefit.

Physic and Philanthropy is a history marking the fiftieth anniversary of the Wellcome Trust, the largest philanthropic organization concerned with medical research in Britain. There is much to celebrate, although the first Trustees may not have thought so during the early years. Indeed, the Nobel laureate and chairman of the Board of the Trust from 1938 to 1960, Sir Henry Hallett Dale, is quoted as making some unflattering remarks about the Trust's benefactor and his interest in archaeology, medical history and the collection of artefacts which now rests in the Science Museum as the Wellcome Museum of the History of Medicine. The first 15 years of the Trust were occupied by keeping the business afloat during difficult times, arranging for the disposition of archaeological remains — but giving away only modest sums for the purposes of medical research.

A. Rupert Hall, a historian of science, has written the bulk of the book. The second, smaller part, which describes the support provided in various areas of interest to the Trust, is contributed by B.A. Bembridge, an assistant director of the Trust since 1969. Perhaps it is just as well that there was little to distribute in the early years, for there was no clear policy from the Trustees as to what ought to be supported. As Hall notes, "no one could describe [their research policy] as highly imaginative, and its innovative fruits were at best indirect." The Trust's grants were heavily weighted to buildings and equip-

ment, constituting 38 per cent of expenditures from 1936 to 1970.

Fortunately, Lord Piercy, who succeeded Henry Dale as chairman in 1960, just as funds began to increase, saw the need for a policy to guide the Trust's benevolence. (After 1970 only 3 per cent went into buildings and equipment.) The shift towards a more rational operation was nudged into place by the first scientific secretary, F.H.K. Green, but given real form and direction by Peter O. Williams, who joined the Trust in 1960 and has been its Director since 1969. Dr Williams's

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Philanthropist and product — Henry Wellcome in 1930, and the Kepler Cod Liver Oil that was one of Burroughs, Wellcome's early successes.

chapter "Past and Future" provides the book's most succinct summary of the Trust's work, and readers with limited time could profitably direct their attention to this chapter alone.

Henry Wellcome's long-standing interests in the neglected subjects of tropical medicine and the history of medicine and science have been pursued as he instructed. According to this account, Sir Harold Himsworth of the Medical Research Council virtually handed research in tropical medicine over to the Wellcome Trust during a lunch at the Athenaeum Club in 1961. The documentation of the Trust's activities in this field is particularly illuminating, and it highlights the value of long-term support of competent investigators in the tropics. Trust-funded projects range from support of the research of Foy and

Kondi in East Africa in the 1930s and 1940s to the more recent studies by David Warrell on the pathophysiology of cerebral malaria in Thailand. Support by the Trust for clinical sciences, buildings, equipment and basic sciences is also well documented.

Sometimes the details of the Trustees' actions become tedious. But it is important to the history of the Trust that the Trustees wrestled early with the problem of how to obtain more capital for the business (the Wellcome Foundation) and also to diversify the Trust's source of income. Both could be accomplished by selling shares to the public. Trustees first considered this solution in the early 1960s but only finally acted on it in 1986. Part of the delay revolved around whether this violated the spirit if not the letter of Henry Wellcome's will. In any case, 25 per cent of the business is now held by the public through shares in the holding company, Wellcome plc. The Trust received £200 million from the sale, which protects it from sudden down turns in the pharmaceutical industry. The move also makes nationalization (a greater concern in the 1960s) less likely.

The Trust's spending reached £35 million in the two years ending in 1984. With the sale of stock and, more recently, the licensing of azidothymidine (AZT) for the treatment of AIDS, its budget is likely to increase. It is expected that the Trust will have available a sum approximately equal to that expended annually by the British Medical Research Council, emphasizing the importance of Wellcome to medical research in Britain. (Readers in the United States will recognize the Trust as the source of the Burroughs-Wellcome Fund, a more homeopathic dose of Wellcome philanthropy. The Fund was created in 1955 when Sir Henry Dale and the president of the American subsidiary, William Creasy, decided to take advantage of the US tax laws allowing industries to establish charitable funds from money that would otherwise go to the Internal Revenue Service.)

Active scientists in Britain will be interested in this careful documentation of Wellcome's enviable record. But more than as a guide to current grant-seekers, the book shows how and why some of the major medical research projects of the past 50 years were supported, and how the Wellcome Trust became a powerful force in science and medical research — a realization of Henry Wellcome's wish to serve mankind. Following a difficult beginning the Trust ends its first 50 years on a high note indeed; one hopes that the next 50 will be equally successful. □

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Great achievements, difficult times

Israeli science has made remarkable strides in a short time. More will come, but there is a danger that Israeli science will be overwhelmed by security problems, both economic and military.

JUST 39 years old, Israel has confidently asserted its claim to be taken seriously as a force in international science. To be fair, Israeli scientists have shown themselves capable of valuable contributions across the spectrum. But having taken its seat at the table reserved for the senior players, Israel now must renew its commitment to basic science, or find itself left behind — or, worse, benignly ignored — as scientists from more affluent countries vigorously forge ahead on research that may seem too expensive for Israeli pockets.

Israel has grown up with a strong science community. Chaim Weizmann, Israel's first president, was also the first president of the scientific institute at Rehovot that bears his name. Weizmann believed that science would have to be one of the pillars upon which the state of Israel would rest, for surely there were and are few natural resources on which Israel can count for economic prosperity. From the first day of independence, Israel boasted three universities, two entirely devoted to science and engineering. Today there are seven universities serving a population just over 4 million. But a country and a culture so used to its commitment to science and learning may be at special risk in taking that for granted. Universities are racking up alarming deficits, students are striking for lower tuition fees, able scientists are being coaxed away to more comfortable research climates and the whole enterprise of basic science has suffered from a decade of financial neglect.

Although fiercely independent, Israel has relied from the start on the generosity of wealthy Jews throughout the world to support all strata of its society, especially science. There is scarcely a building in Israel that does not bear the name of some

wealthy patron. But Israel is no longer in desperate need of buildings: it needs operational cash. US foreign aid has been particularly crucial, amounting to \$3,000 million a year, or approximately 12 per cent of Israel's gross domestic product. But where do researchers go for money to operate their laboratories? They go abroad. For example, the US National Institutes of Health will provide \$3.6 million in grants and contracts to Israeli researchers this year. Domestically, the Binational Science Foundation is the largest source of funds for basic research, shelling out about \$8 million a year, but to qualify for a grant one must have a bona fide US collaborator. The Israeli National Council for Research and Development gets only about \$2.7 million from the Israeli government, although it does distribute other money, mostly from foreign sources. There is an effort now afoot in Israel to establish an analogue of the US National Science Foundation, with an endowment — provided by US philanthropists, matched in some fashion by the Israeli government — that would be wholly controlled by Israelis. This is a good idea, but the philanthropists have not jumped at it. And it would be only a small start at weaning Israeli scientists from their dependence on foreign money.

In pushing toward economic independence — or what will pass for economic independence given continued US support — Israel has concluded (as have others) that it must put its resources into applied research, projects that will give Israel a market niche that it can profitably exploit. The \$80 million budget of the chief scientist of the Ministry of Industry and Trade for supporting applied research puts all sources for basic research to shame. Although it is true that this money

will help companies in the science-based industries to grow and develop, it is a fallacy that it will inevitably make more successful the companies so helped. Scitex and Elscint, two Israeli companies renowned for their high technology products, the one in colour publishing and the other in medical diagnostics, have suffered large financial setbacks in the past year or two. These blows have not come from technological shortcomings, or from a failure to spend enough on research, but from poor management. The two companies were also uneasy in the big leagues of international sales and found the competition rougher than expected.

The pendulum has been swinging against basic research since the mid-1960s, when a commission headed by Ephraim Katchalski-Katzir recommended that Israel should spend more on applied research. The time has now come to reverse the swing. But who in the Israeli coalition government is going to give the needed push? Somewhere along the way, basic research began to get a bad name in Israel. The Likud government was especially anti-intellectual in its attitude towards the universities, as if somehow being a professor was not as honorable as Jewish culture had held. Shimon Peres, the first prime minister at bat in the present coalition's approach to the government of Israel, was friendlier, but could only halt the previous dizzying decline in support for higher education. Scientists who once enjoyed prestige if not high salaries are beginning to lose some of that prestige to those to whom society pays higher salaries. Although the numbers leaving universities for the "good life" in industry — either in Israel or abroad — is still a trickle, one senses that it has the makings of a rivulet if not a major flood. And for a small country, even a trickle can be devastating.

Many of Israel's difficulties in the allocation of limited resources are the same as those now encountered elsewhere, but Israel has its own special problems. Money for basic research will probably always be tight in a country that spends nearly 60 per cent of its research budget on defence. A solution of its political problems would give Israel the luxury of supporting its strong scientific community generously. The results of such support would not be disappointing. Is that opportunity not another prize to seek in an accommodation between Israel and its neighbours? □

Acknowledgements

EXCEPT where indicated, this survey of Israeli science has been written by *Nature's* Washington News Editor, Joseph Palca, who wishes to thank Nechemia Meyers and his staff at the Weizmann Institute of Science, Carol Cook at Tel Aviv University, Jerry Barach and David Lavie of Hebrew University, and Haia Galai of Ben Gurion University, for their help in arranging interviews, sometimes at very short notice.

This survey is necessarily idiosyncratic and incomplete. After two weeks in

Israel, Palca says he felt as if he had been invited to a lavish smorgasbord, but with only a small spoon to sample the dishes; what follows is an attempt to represent the flavour of Israeli science.

A note on spellings. There are no uniform rules for rendering Hebrew names into English. Spellings used are those preferred by individuals; apologies are due to those whose names are spelled otherwise.

Today the Israeli shekel is worth \$0.63. Many in Israel express financial data in US dollars, not shekels, because of the recent volatility of that currency, whence the practice in what follows. □

History

Constant struggle for survival

ONLY in the past twenty years has a generation grown up not knowing any home but Israel. For much of the country, the decision to live in Israel involved a choice, to leave one home to help create another. Zionism means different things to different people. Although far from a homogeneous movement, Zionism historically had as its central objective the return of the Jewish people to their historical home, and the creation of a Jewish state. Zionism almost by its nature has required a willingness to put up with hardships as part of the price one must pay to achieve that goal.

Jews from Eastern Europe began a move back to Palestine in the late nineteenth century. At the time, the region was under the control of the Turkish Ottoman empire. In 1897, the first World Zionist Congress, organized by Theodor Herzl, met in Switzerland, starting a political drive for a Jewish state.

The First World War brought Britain into direct conflict with Turkey, and in 1917 the British foreign secretary Arthur James Balfour wrote to the then Lord Rothschild promising British support for the creation of a Jewish homeland in Palestine — the Balfour declaration as it became known. At the end of the war, under a mandate from the League of Nations, Britain took control of Palestine, Iraq and Transjordan (later Jordan).

After the Second World War, pressure for an independent Jewish state grew, in part in response to the Nazi murder of Jews. In 1947, Britain decided to allow the infant United Nations to solve the question of what to do with Palestine. In November 1947, the United Nations General Assembly adopted a plan, which was supported by the United States and Soviet Union but opposed by Arab states, for partitioning Palestine, creating Jewish, Arab and international zones. On 14 May 1948, Britain ended its mandate and David Ben-Gurion, soon to be Israel's first prime minister, read the Declaration of Independence. Chaim Weizmann became Israel's first president, starting a tradition of scholar-presidents.

The birth of Israel was not easy, as the neighbouring Arab nations fought to prevent implementation of the UN partition. After a year of fighting, Israel had acquired more territory, but ceded control of the Old City of Jerusalem to Jordan.

Armed conflicts with neighbours have been frequent in Israel's brief history,

sporadically escalating into full-scale war. In 1956, Israel invaded the Sinai and Gaza Strip, as part of a coordinated effort with Britain and France to block Egyptian nationalization of the Suez Canal. Under pressure from the United States and the



Soviet Union, the invading forces withdrew from Egyptian territory in 1957, and UN troops were stationed in the area.

In 1967, tensions with Syria to the north and Egypt to the south reached a boiling point. After Egyptian President Nassar ordered UN troops out of the Sinai, Israel, fearing an impending invasion, struck against Egypt, destroying its air force. In the Six-Day War, Israel took control of the Golan Heights to the north, the West Bank, the Gaza Strip along the Mediterranean, the Sinai, and the Jordanian-held portions of Jerusalem.

The Six-Day War gave Israel vastly expanded territory, and a large non-Jewish population with which to contend. In November that year, the UN Security Council passed Resolution 242, calling for a withdrawal of Israeli forces from all occupied regions and recognition of "the sovereignty, territorial integrity and political independence of every state in the area . . ." This document, intended as a basis for a lasting peace, has not been

endorsed by all parties in the Middle East.

In October 1973, Egypt and Syria launched a coordinated attack against Israel on Yom Kippur, the Jewish Day of Atonement. Caught off-guard, the Israeli army nonetheless traded territory with Egyptian troops around the Suez Canal, and drove Syrian troops back toward Damascus.

A significant step toward peace in the Middle East came in 1978 when Israeli Prime Minister Menachem Begin — whose Likud party had formed the government the previous year — and Egyptian President Anwar Sadat signed the Camp David accords, settling the conflict between their two states and establishing a framework for peace in the region. Although relations between Egypt and Israel have not become cordial, the two countries have been at peace, and in 1982 Israel completed its withdrawal from the Sinai.

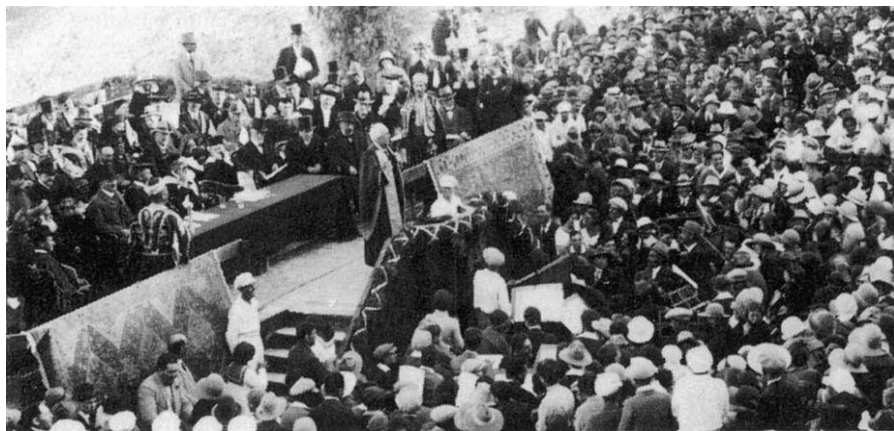
Relations with its neighbours to the north have not improved since 1967. In 1981, Israel extended its law and jurisdiction to include the Golan Heights. In 1982, avowedly to prevent further terrorist attacks on northern settlements, Israel invaded Lebanon. Always a controversial action at home and abroad the invasion never completely achieved its military objectives, and Israel has withdrawn most of its forces.

In 1983, Begin resigned as Prime Minister, and his successor Yitzhak Shamir called early elections in 1984. Since then, a coalition between the Likud and Labour parties has ruled, with Labour's Shimon Peres serving first as prime minister, and in 1986 Likud's Shamir taking over.

Since 1967, Israel has failed to find an acceptable scheme for withdrawing from the West Bank. Begin's Likud government actively supported Jewish settlements in the region, but by doing so damaged plans to develop the Negev. Any permanent peace settlement will have to make an accommodation with the non-Jewish Arab population of the West Bank, but no plan acceptable to a majority



First of Israel's scientist-statesmen, Chaim Weizmann. He was director of the British Admiralty laboratories in 1916–19, before moving on to the Daniel Sieff Research Institute.



Lord Balfour opens the Hebrew University in April 1925.

of Israelis, let alone one that would be satisfactory to its Arab neighbours, has yet been proposed.

Israel's economy has been enjoying a modest boom after a few years of dire problems. Inflation as high as 445 per cent in 1984, dropped to around 20 per cent last year. Business growth has picked up, and unemployment now stands at an accept-

Research policy

Government by many pockets

AUTHORITY for setting policy and distributing money does not reside in one individual office of the Israeli government, but is rather spread throughout the various ministries. This decentralized organization results from the recommendation of a commission that was chaired by Ephraim Katchalski-Katzir, a professor at the Weizmann Institute of Science and later the fourth president of Israel (see chart below).

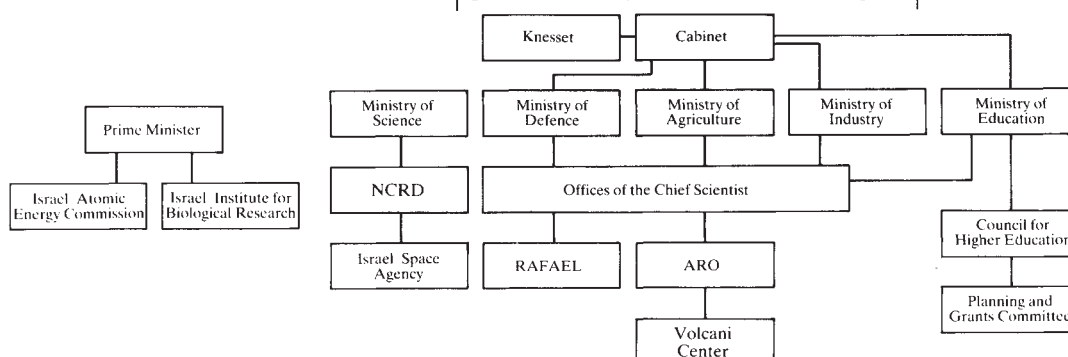
In 1968, the commission urged that an office of the chief scientist be created in each ministry. The idea was for each ministry to use its science budget to further its own goals, rather than have one pot of money for all scientific research. The Katchalski-Katzir commission also recommended that the National Council for Research and Development (NCRD) be vested with the power for establishing science policy for the whole country, but this recommendation has never been implemented.

able 5.7 per cent.

But problems persist. Despite real growth in exports, the non-military trade deficit is widening. Although the government budget has boasted a net surplus in the past two years, the sudden withdrawal of the annual \$3,000 million in US foreign aid would certainly plunge Israel back into a deep economic hole. □

Some ministries have come to play important roles in the pursuit of Israeli science. The chief scientist for the ministry of industry and trade, for example, provides government support for start-up companies, and has been actively engaged in forming international cooperative agreements in industrial development and technology. In the ministry of agriculture, the Agriculture Research Organization (ARO) sets policy for agricultural research, both at the government-run Volcani Center and at academic institutions.

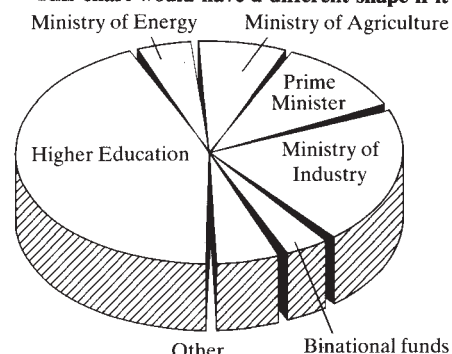
Energy's chief scientist also supports university and industrial research although recent policy has supported applied rather than basic research. The defence ministry conducts its research activities both through its wholly owned armament development authority (RAFAEL) and the Israel Military Industries, as well as supporting projects both in private industry and universities. Surpris-



Research spending

The chart below shows the most recently published figures for the Israeli government's spending on research. The total amount spent on non-military research and development in 1983/1984 amounted to \$223.9 million. Higher education gets the largest slice of the non-defence pie. Money for higher education is distributed by the Planning and Grants Committee (PGC), a body essentially independent from the government. Although the amount distributed by the PGC is substantial, representing approximately 30 per cent of the total for higher education, it is somewhat misleading. Universities have been forced to use some of this money for basic maintenance and overhead.

This chart would have a different shape if it
Ministry of Energy Ministry of Agriculture



included government spending on defence. Defence research and development spending amounted to \$257.9 million in 1983/1984, or 53.53 per cent of the total government spending on research and development.

Israel spends approximately 3 per cent of its gross domestic product on research and development, high by international standards.

ingly, the chief scientist in the ministry of health has a tiny budget, and does not have a major role to play in biomedical research.

The prime minister's office has retained control of the Israel Atomic Energy Commission (IAEC), which in turn runs the two experimental nuclear reactors at Dimona and Soreq. Also under the prime minister is the Israel Institute for Biological Research (IIRB) in Ness-Ziona. In addition to conducting both basic and applied biological research, IIRB markets some of the fruits of the research that is performed there.

In 1982 the government created the science ministry, and moved the NCRD into it. The new ministry has served mostly an advisory capacity, although it does have a small budget. The Israel Space Agency is also a part of the science ministry.

Money for higher education goes to the Planning and Grants Committee through the independent Council for Higher Education. PGC is nominally part of the education ministry, but for the most part acts independently. □

Binational funds

US – Israel research combination

THE United States–Israel Binational Science Foundation (BSF) is a unique body. Although both countries have had numerous bilateral agreements for scientific exchanges, BSF is the first organization to have its own independent board of directors that decides how to allocate grant money. BSF supports basic research across the scientific spectrum, although the emphasis has been on the life sciences.

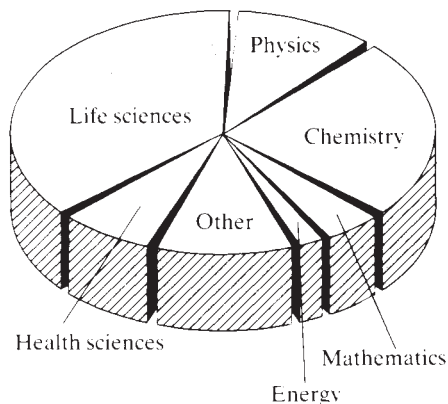
Zeev Rotem, executive director of BSF, says the foundation is able to sup-

port about one third of the 400 grant applications it receives each year. He estimates that more than half deserve support. "We don't support those that are good," he says. "We only support those that are very good to excellent, and even then we can't support them all."

Grants are awarded based on scientific merit, the strength of the collaboration between US and Israeli partners, and the interest of both governments in the project. Rotem says he also tries to support younger people just starting out in their careers. All projects must involve collaboration between scientists from both countries.

The United States and Israel established BSF in 1972, with each contributing the equivalent of \$30 million in shekels. That endowment increased by \$20 million each in 1984, bringing the total to \$100 million. The amount BSF can award in grants varies with interest rates. This year it should be around \$8 million.

There are two other US-Israel funds; BIRD-F, the Binational Industrial Research and Development Fund, and BARD, the Binational Agriculture Research and Development fund. Both have



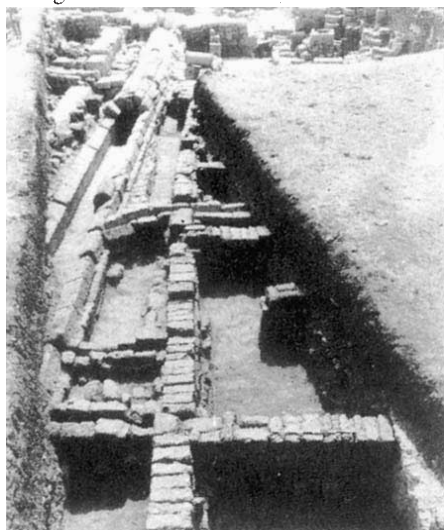
How BSF has allocated its resources.

Archaeology

Embarrassment of riches

If Israel is lacking in fossil fuels, water and other natural resources, there is one resource in which it is extremely rich: antiquities. For archaeologists, Israel presents a seemingly endless selection of research opportunities.

Ephraim Stern is a professor at Hebrew University conducting an excavation at Dor, just south of Haifa along the Mediterranean coast. Dor is one of the few surviving sites of Phoenician settlements along the eastern Mediterranean.



Phoenician civilization flourished from about 1,200 BC until 146 BC, when the Romans destroyed Carthage. Stern's excavation at Dor covers 75 acres, and goes to a depth of 17 metres (see figure). He has found artifacts as old as 2,000 BC. The excavation at Dor is both for research and teaching. Each summer, several hundred students from Israel and abroad arrive to participate in the dig.

Archaeology is taken quite seriously by the Israeli government. When Israel gained control of the old city of Jerusalem, the Department of Antiquities quickly established that no building could take place without permission. Stern says that sifting through the foundations the Jewish Quarter provided a once in 2,000 year opportunity to see what was going on in one third of the old city. Today, beneath shops and private dwellings, streets from the days of Herod are beautifully preserved. One enterprising resident opened a museum to display the relics that were found in the foundation of his own house.

If other disciplines sometimes suffer from a scarcity of people, that is not the case for archaeology. "Here there is a large concentration of people dealing with all different fields of archaeology", says Stern. "That is what makes us different. And, of course, the material is here." □

endowments of approximately \$110 million. BIRD-F also receives some revenue from royalties from commercial projects it has helped support.

Last June, at a BSF governor's board meeting, it was suggested that the endowment for BSF should be doubled, by contributions from each country of \$10 million a year for five years. Although the suggestion met with lukewarm support, Rotem is not overly discouraged. He says the call to increase the endowment in 1975 was not heeded until 1984. □

Yeda means knowledge

Universities in Israel have decided there may be a profit in promoting some of the research done by their faculties. Since all Israeli universities are non-profit institutions, a way had to be found to avoid losing favourable tax status while reaping the rewards of marketable research.

At the Weizmann Institute of Science in Rehovot, the answer is called Yeda — the Hebrew word for knowledge. Yeda is a trust that retains the rights to commercial profits from patents developed at the Weizmann. The chairman of Yeda is a member of the Weizmann faculty, now biophysics professor David Mirelman.



Mirelman — looking for development

"A research institute is good in research, not development", says Mirelman. Yeda has sought companies with commercial expertise with which to establish partnerships, receiving equity in joint ventures for providing the know-how. Yeda's most successful partnership was with the pharmaceutical company Miles, but that ended in 1983, with Yeda buying out Miles (see page 580). Last year, after much soul-searching, Yeda sold its share in the company to Makor Chemicals, a subsidiary of the US chemical company Sigma. Mirelman says Yeda was reluctant to sell a successful Israeli business to foreign investors, but the alternative was to become more involved in international marketing, an unappealing prospect.

In addition to these intermediary holding companies, most universities have established science parks near their campuses. Universities have become aware that their faculties can be attractive to the commercial world. □

Israeli scientists

Intangibles maintain a hold

PART of the attraction of working as a scientist in Israel is the status. Learning and knowledge have been traditionally important in Jewish culture. Former Israeli president Ephraim Katchalski-Katzir says the cliché that every Jewish mother wants her son to be a doctor or a rabbi is still true. David Horn, a physicist at the University of Tel Aviv, says that, when visiting the United States, he has none of the positive feeling from being a professor that he is used to. "That is part of the reason we like to come back home", says Horn.

But that exalted position may be slipping. Ephraim Arazi, chairman and chief executive officer of Scitex Corporation, Ltd, a manufacturer of state-of-the-art printing equipment, says that stock options and profit sharing have raised the image of people whose intellectual pursuits are directed more towards profit than education. If he is correct, then such a change may be the harbinger of a major shift in the foundations of Israeli society. Ahad Haam, a Zionist in the first half of this century, argued that it was not enough to create a political state of Israel, but that true Zionism includes maintaining Jewish cultural heritage. Alexander Keynan, former vice-president of Hebrew University, believes a goal of Zionism ought to be the pursuit of a world centre of excellence, something that has not yet been achieved. If Haad's vision is taken at its face value, universities are not just a luxury or a key to economic prosperity but a critical part of Israel's reason for being.

Irun Cohen, a biologist at the

Weizmann Institute, says there is no Jewish culture that does not see learning as an end in itself. Transmitting that learning has seemingly been a driving passion for Israeli scientists. They have been incredibly prolific; individuals publishing books or papers in international scientific journals in the natural and engineering sciences in 1984 numbered 50 per 10,000 in the labour force, 50 per cent more than the next closest country, Switzerland.

But there are many disadvantages in working as a scientist in Israel. Every able-bodied person must spend up to 40 days per year in the army. Take-home pay for full professors amounts to little more than \$1,200 per month, a small amount in a country with a high cost of living and double-digit inflation. Teaching loads at the larger universities are heavy, and with only seven universities, the options for moving on within the country are few.

Faced with these problems, together with limited equipment, sparse research money and geographical isolation, many Israeli scientists hear the siren call from well-heeled research institutions in the United States and Europe. An Israeli scientist can make as much money in two months at a summer appointment at an Ivy League university as he will the entire rest of the year back home. A summer spent in a US laboratory may provide the only opportunity of the year to collect experimental data.

In order to offset inevitable losses of people, Israel has relied on a "reverse brain drain". Immigrants have traditionally brought with them skills important to



One of the grand old men of Israeli science, and a former president, Ephraim Katchalski-Katzir.

the state, and scientists have been no exception. But the numbers flowing into Israel have been small in recent years, and there is a fear that they are not enough to match the outflow.

Nevertheless, some find the attraction of Israel irresistible. "As a Jew, I thought I should be here," says Charles Hexter, deputy managing director of the biotechnology company Bio-Makor. "I don't have to dig a ditch or build a road to contribute to Israel", he adds. "My being here is not crucial to the state, but it gives me satisfaction."

Scientists stay in Israel for both complex and simple reasons. Family, philosophy, commitment, religion and faith all play a role. Ben-Gurion University professor Zamik Rosenwaks returned to Israel after spending several years on the faculty of the University of California at Santa Barbara for reasons that are not complicated to him: "It is as simple as the question, 'where do you feel at home'?" □

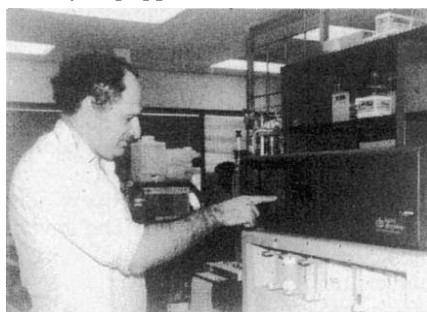
Working at a home away from home

THE case of Joseph Schlessinger, Jossi to his friends, is an example of an Israeli scientist drawn away from home. Schlessinger is a tenured professor at the Weizmann Institute of Science in Rehovot. Three years ago, with colleagues from the Imperial Cancer Research Fund and Genentech, Schlessinger was the co-author of a paper showing the similarity between an oncogene from a tumour virus in birds and the gene encoding a receptor for epidermal growth factor (*Nature* 307, 521; 1984). The work drew instant recognition, and by 1986 had become one of the most frequently cited papers in cancer research, according to *Current Contents*.

Already well established, Schlessinger says he was flooded with job offers. With a sabbatical year coming up, he accepted a post at Meloy Laboratories near the US National Institutes of Health in Rockville, then a division of Revlon, but later sold to the pharmaceutical company Rorer.

Following the sale, Schlessinger became Research Director at Meloy, now called Rorer Biotech Inc. He has extended his stay in the United States by receiving a two-year leave of absence from the Weizmann, although he still maintains a small research group in Rehovot.

It is by no means certain that Schlessinger will return to Israel. Even the most lavishly equipped laboratory by Israeli



Would 'Jossi' have such good facilities in Israel?

standards pales in comparison to the facilities at Rorer Biotech and his research budget is large by any standard.

Schlessinger says he wants to return. He chose a job in industry instead of a university position so that he would not have to accept tenure elsewhere. He is stung by criticism that he "sold out" by going abroad. He says he retains close ties with friends and colleagues in Israel, and tries whenever possible to use his resources to help them out. He would like to work with Rorer to open opportunities in Israel where he feels that there is more than sufficient talent to do high quality work comparable with that in the United States.

But Schlessinger worries that returning may be difficult. If he succeeds in bringing private research money with him, arriving with a superbly equipped laboratory and a salary way above Israeli standards will certainly cause problems. Giving up the research advantages he now enjoys would seem a bit like cutting off his nose to spite his face. □

The universities

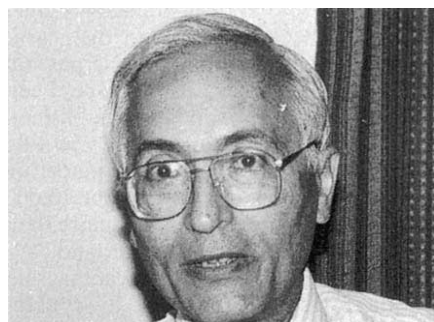
Keeping higher education on track

EACH year, when the time comes to determine how much government money the universities in Israel will get, Jacob Ziv sits down at his personal computer and makes the calculations by himself. As chairman of the Planning and Grants Committee (PGC), Ziv needs only a small computer to arrive at the division of his budget of some \$230 million. But working out the formulae to derive those numbers is a full-time, year-long task, fraught with political arm twisting and financial haggling.

All the universities in Israel are private, but each receives a substantial portion of its operating budget from the PGC. The PGC was established 14 years ago, modelled after the British University Grants Committee. The PGC chairman is usually an academic. Ziv is a professor of electrical engineering at the Technion. His predecessor, Haim Harari, is a professor of physics at the Weizmann Institute.

PGC has six members; four academics and two from the public sector. The committee's authority comes from the Council for Higher Education, a body chaired by the Minister of Education and Culture but otherwise independent of the government. PGC members must receive government approval.

The committee has exclusive authority to distribute the government's budget for higher education, and is supposed to oversee the entire higher educational structure in order to prevent "superfluous duplication" and encourage economy. Ziv



Ziv and microcomputer plot the course.

says that the PGC is also supposed to advise the government in formulating its budget for higher education — but "they never listen to us, of course".

Ziv says that his basic formula divides the money available between universities so that three-quarters of a university's allocation is based on enrollment figures and one quarter on research activity. But he emphasizes that it is the volume of such activity, and not the quality that determines the spending.

Although the PGC does not tell a university exactly how to spend its money, it must see and approve each university's budget. Although in most cases the government provides the largest single block of money to the universities, they also rely heavily on patrons scattered throughout the world. Even a relatively young university, such as Ben Gurion, has "friends" committees in nine countries, including eight chapters in the United States. Technion, a much older institution, has supporting societies in 22 countries.

The PGC has sought to encourage universities to use these friends associations to establish endowment funds. Starting in 1983, PGC has paid 7 per cent interest on all new endowment funds brought into the country. By 1987, Ziv estimates that 10 per cent of the PGC budget will go to these matching payments.

When the PGC was established, Israeli universities were relatively prosperous. But from 1973 on there has been a steady decline in government support, a decline that most believe went well past the point that would have allowed the universities to avoid their current difficulties.

In 1983 a crisis point was reached. Government support took a sharp dip, and inflation began to run out of control. Universities had always felt hostility from Menachem Begin's Likud government, and this feeling intensified. The government began holding up payments to universities, and when the money did arrive, triple-digit inflation had eroded its initial value.

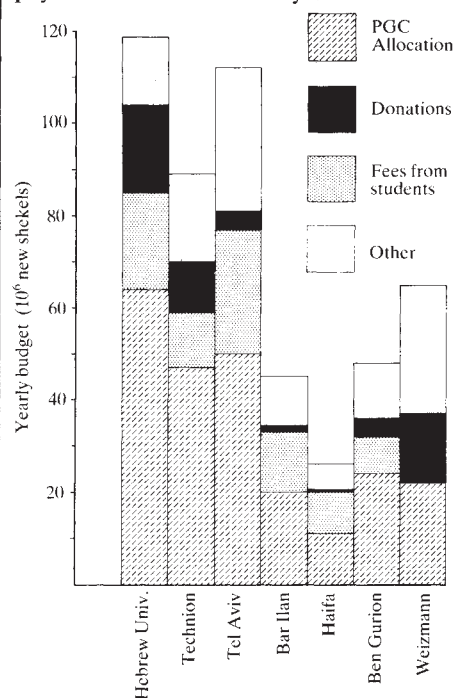
As PGC chairman at that time, Harari

wrote that "before the opening of the 1984/85 academic year, the PGC made it clear to all concerned that it would not and could not continue to carry out its duties unless the gap between the income of the universities and their expenses was closed". The government did respond by providing additional money, although not as much as had been cut the previous year.

Since 1984, the government support for universities has stopped going down, and there may even be a slight upswing in real terms. "The government understands in some vague way the importance of research", says Harari. But he sees little evidence that such understanding is being translated into acts. Harari worries that the effects of neglecting the universities are only now beginning to be felt, and will spread to all segments of the society in the next decade.

University facilities and equipment in particular need strengthening. The PGC has established a special fund to force universities to buy new equipment and books. With personnel costs accounting for up to 70 per cent of a university's budget, the tendency often is to cancel equipment purchases to save money.

The PGC has also put forward a four-year plan to the cabinet that would raise payments to universities by \$17 million a



The budgets of Israel's seven universities for 1985-86.

year for the next four years. If a proposed tuition increase goes through, the government would not actually have to contribute extra money to the PGC for two years.

Ziv is convinced that universities can learn to keep their financial houses in order. It takes leadership to decide whom to support in tough economic times, says Ziv. Money must be found to allow the brightest lights to continue to shine. □

Tuition troubles

DURING the current academic year, students have repeatedly gone on strike to protest at tuition fees which, they say, are higher than they can bear. Students now pay about \$1,380 per year, only \$800 of which is for tuition, the rest a special tax. The students are asking that the special tax be eliminated. The Minister for Education is proposing the total package be lowered to \$1,120 a year. The Planning and Grants Committee would like to see tuition set at \$1,680 per year, with fellowships for low-income students. The universities have supported a plan that would set the annual tuition at \$1,850.

Students in Israel tend to be older than their counterparts in the United States or Europe, since most spend from age 18 to 21 in the army. Many have already started families of their own before beginning university education. Supporting higher tuition will not be a politically popular stance, especially if early elections are called. Students represent 65,000 votes. □

Weizmann Institute

A major national resource

THERE are a relatively few first class science institutions in the world, says Ephraim Katchalski-Katzir, former president of Israel, but the Weizmann Institute of Science in Rehovot is one of them. Although he is no doubt prejudiced from his long association with the institute, many would accept his assessment. The Weizmann has managed to produce some of the brightest stars in disciplines ranging from molecular biology to artificial intelligence. Although beset by the same financial burdens now plaguing all Israeli universities, the institute's problems are not nearly as severe, and scientists there continue to enjoy relative prosperity. But there is a growing fear that without some changes, even the Weizmann will feel the chill winds of financial neglect.

Unlike Israel's other universities, the Weizmann Institute has no undergraduates. The student population consists of approximately 330 doctoral and 170 masters degree candidates. Total faculty, including postdoctoral fellows, numbers about 600.

The institute began life in 1934 as the Daniel Sieff Research Institute, named for the 17-year-old son of Israel Sieff, from the family that owns the British department store chain Marks and Spencer. The Sieff Institute's first president was Chaim Weizmann, a chemist from Russia who worked in the United Kingdom during the First World War. Weizmann worked with polycyclic compounds, and discovered a bacterium that

could transform starch into acetone and butanol. The acetone dissolved the nitro-cellulose powder used in propellants. Katchalski-Katzir says Weizmann's friendship with Lord Balfour during the war played a role in the Balfour declaration promising a Jewish state.

Professor Herman Mark and Professor Ernst David Bergman played pivotal roles in determining the direction for the Sieff Institute. Following the Second World War, the institute continued to expand, and in 1946 the cornerstone was laid for a new institute, named after the 70-year-old Chaim Weizmann. But before the new institution could formally open, the war of independence intervened. Katchalski-Katzir, then a young scientist at the institute, says that the younger researchers wanted to turn to applied projects to help the war effort. But Weizmann kept them pointed toward basic research. Katchalski-Katzir says Weizmann believed that basic science had to form one of the foundations of a new state.

In 1949, the Weizmann Institute formally opened its doors. Today expansion has slowed considerably. Weizmann President Aryeh Dvoretzky says the institute still plans to grow, "but in a gradual fashion." The major building project is a new \$10 million solar tower, an object of some controversy (see page 581). The institute's operating budget is some \$60 million, according to Dvoretzky. The government provides approximately one third of this amount, with another third from contracts and grants and the rest from donations and deficit financing. Dvoretzky says last year the Weizmann's deficit amounted to \$750,000, but this year that amount could reach \$3 million.

As a small institution devoted more to research than to teaching, the Weizmann has a flexibility not shared by other Israeli universities. Dvoretzky explains that Weizmann can concentrate its resources where they can make the greatest impact. Hebrew University cannot close a department if it begins to founder, but Weizmann can, says Dvoretzky. The institute is also prepared not to be "fair" in allocating its resources.

"The guiding word is excellence", says Dvoretzky, adding that the institute will pursue the best work "on a non-egalitarian basis". This push for excellence has

prompted the institute to neglect some basic maintenance of its physical plant in order to keep more money available for research.

Michael Sela, a biologist who preceded Dvoretzky as president, says chronic financial pressures have forced the institute to make maximal use of all its instrumentation. As an example, Sela says the institute has only two cell sorters that must be used around the clock to supply the needs of all the researchers on campus. Of course not all research equipment is oversubscribed. Many individual researchers have managed to assemble extremely well-equipped laboratories that more than meet their individual needs.



The Daniel Sieff Research Institute in Rehovot, was the core of the Weizmann when it was established in 1949.

Although Sela's evaluation of equipment shortages is most likely correct for the larger picture, a tour of laboratories reveals that even major pieces of equipment such as an electron microscope can be found sitting idle.

The institute has five faculties; biology, biophysics-biochemistry, chemistry, mathematics and physics. There is also the Feinberg Graduate School where the department of science teaching is located (see page 577). There are also some 13 research institutes on campus that cut across departmental lines.

The strengths of the Weizmann Institute are spread across many disciplines. There are strong faculties in molecular, cell and developmental biology, genetics, structural chemistry, theoretical physics, applied mathematics and artificial intelligence to name but a few. In a way, says Sela, the Weizmann has been a victim of its own success. With salaries effectively fixed by the Israeli government, the Weizmann cannot sweeten the pot to keep its brightest stars even if it could afford to do so. Many faculty are confronted with tempting offers from industry and universities overseas, and quite a few — like Shimon Ullman, below — have begun to divide their time between the Weizmann and institutions abroad.

But the Weizmann also generates strong loyalties. Sela says that since 1949

The rate for the job

STRICTLY speaking, faculty salaries are determined by bargaining between the universities and the teachers. But in practice, since the ministry of finance must approve any salary arrangement, the government plays a critical role in establishing salaries.

Salaries are uniform throughout Israeli universities. Ziv estimates that the take-home pay of a full professor is about 2,000 shekels per month, or about \$1,260. A sore point with students who oppose increased tuition payments is the 4,500 shekels annual travel allowance. Faculty are also entitled to one full year's sabbatical after 6 years of teaching. There is a financial incentive to leave the country on these sabbaticals. Faculty salaries leap to 60,000 shekels per year for full professors who leave the country, whereas those who stay in Israel receive their regular salary.

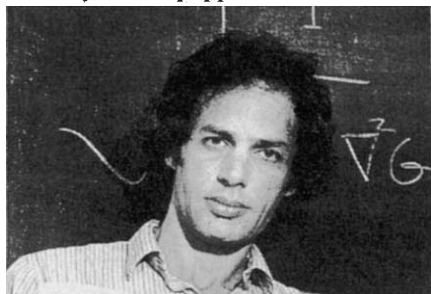
Comparable salaries in industry will be 2.5 to 3 times higher than those at universities. □

Two faces of Israeli research

• **SHIMON Ullman**, director of the Center for Artificial Intelligence at the Weizmann Institute, is considered one of the up and coming stars. He spends about half his time in Israel, the rest of the time at Artificial Intelligence Laboratory at MIT in Cambridge, Massachusetts. He has graduate students at both institutions.

Ullman's work has been on vision, part of a rich vein of artificial intelligence research that goes beyond a mere description of vision and into the underlying cognitive processes.

Ullman's centre at the Weizmann is extremely well equipped. There are five



Symbolics LISP machines, the most popular computer currently available for artificial intelligence research. Ullman says with pride that there are few places in the world with a network of five such machines.

Finance comes entirely from sources within Israel, including some from the research arm of the defence ministry. The Weizmann centre is *de facto* a national resource.

If Ullman faces a problem working at Weizmann, it is the small number of people working in his area. He says his group lacks the critical mass he feels is necessary to maintain momentum. At MIT this is not a problem. Working in Israel forces you to talk more with people outside your area, says Ullman, but on the whole he considers lack of critical mass a liability. His commitment to Weizmann and Israel is more a personal rather than a philosophical one —

only four tenured faculty have left the institute. Although Sela himself is spending the year in the Boston area dividing his time between Harvard, MIT and Tufts University, he declares that "the Weizmann is my ultimate commitment as long as I live."

Slow growth and its small size has meant new faculty face an extremely difficult time getting tenure. For somebody who is outstanding, there will never be a problem, says David Mirelman, who has a chair in microbiology and parasitology. But getting unanimity on who is 'outstanding' is extremely difficult. The tenure committee asks eight outside reviewers to submit letters answering the question "would you have this person as a member of your faculty?" These letters are studied extremely closely, and Mirel-

man says that even the slightest reservation in one of these letters can be damning. For a faculty member who does not receive tenure the blow can be devastating, since academic jobs within the country are scarce.

Ullman, a professor in applied mathematics says "people are not scared away" by the tough fight for tenure. But Ullman, too, wonders "what will happen to the good young scientists who don't make it through tenure?"

Dvoretzky believes that a 10 per cent increase in government support for Israeli universities would go a long way in easing the burdens that they are all now feeling. Whatever the financial pinch, the Weizmann Institute seems destined to remain a fixture in the international scientific scene for the foreseeable future.

he was born in Israel, and most of his family is there. But colleagues say sooner or later he will probably have to take steps to unify his binational existence.

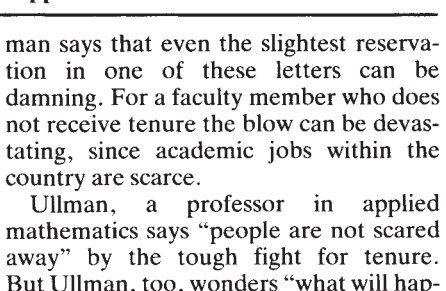
• **IRUN Cohen**, professor of cell biology at the Weizmann Institute, came to Israel as a postdoctoral fellow of the Arthritis Foundation in 1968. He intended to stay

neither at the Weizmann nor in science at all, but he got interested in immunology while in Israel and in the study of autoimmune diseases. He says his was the first laboratory to raise clones of T-lymphocytes for target tissues in the body. "The T-cell approach to autoimmunity grew up here", says Cohen, "and anyone who wants to work on it comes here."

But even with his special expertise drawing visiting scientists, Cohen says foreigners still see Israel as "the end of the line." He says people who visit Israel from the United States will often stop off in Europe on their way back, but rarely will they add a trip to Israel when visiting Athens.

This physical buffer can have a positive effect. Although scientists in Israel will find it difficult to participate in rapidly moving topic areas — AIDS research as an example — Cohen says they are spared from being swallowed up in such mass projects.

Cohen says he enjoys the protection that



comes from being outside the mainstream. He has steady collaborations with scientists in the United States, Denmark and Sweden, and enjoys some measure of financial support from each of those countries.

man says that even the slightest reservation in one of these letters can be damning. For a faculty member who does not receive tenure the blow can be devastating, since academic jobs within the country are scarce.

Tel Aviv University

Looking toward restructuring

YEHUDA Ben-Shaul, the rector at Tel Aviv University, has a large blue notebook that he keeps in the top drawer of his desk. Ask him where the university is heading, and he hauls out the notebook and shows that there are big plans in the works.

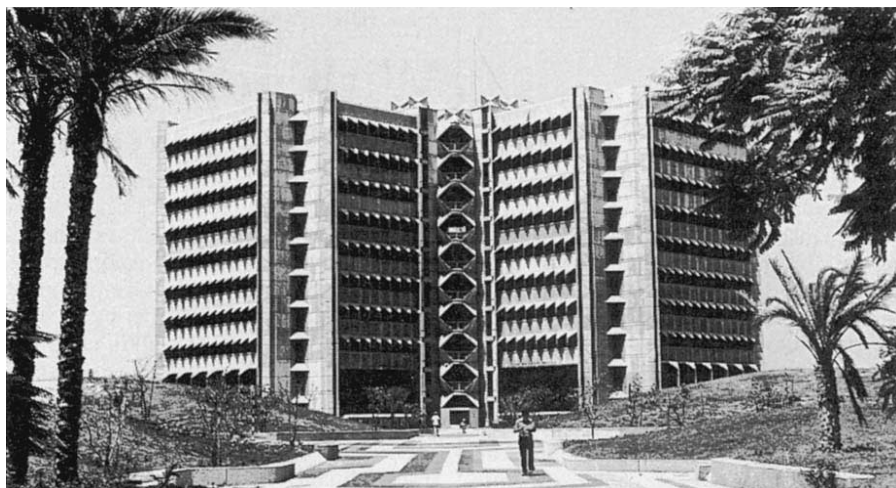
Tel Aviv University is the largest in Israel. There are some 20,000 enrolled students, with approximately 5,000 pursuing graduate degrees. Another 9,000 students in non-degree programmes give the spacious campus at the northern edge of Tel Aviv a bustling, active feeling.

The university grew out of two institutions of higher learning established by the municipality of Tel Aviv in the 1950s. Since its formal establishment in 1956, the university has been growing rapidly, adding faculties in the disciplines of engineering, medicine, law, humanities and social sciences. In 1972 the faculty of natural sciences was divided into the exact and life sciences, a division that persists to this day.

Ben-Shaul has big plans for reorganizing the life sciences. The faculty now has five departments: biochemistry, microbiology, zoology, botany and the newly formed biotechnology department headed by Ephraim Katchalski-Katzir. These divisions made sense in the early days of the university, when teaching was emphasized and interest focused on the flora and fauna of the new country. But as modern biology shifted toward molecular biology and genetics, the traditional department structure became cumbersome, with people working in similar areas of research separated by layers of bureaucracy.

Under the new scheme, there will be two schools in the life sciences. One will focus on molecular, developmental and cellular biology, and biotechnology. The other will encompass ecology and physiology. Ben-Shaul says the university will try to give a special push to biotechnology and cell and developmental biology, adding faculty when possible and channeling what limited resources there are into these areas. Programmes in molecular biology, genetics and neurobiology will continue to enjoy positive support, but classical topics such as field botany and zoology will be allowed to fall into benign neglect. An equally ambitious reorganization is planned for preclinical studies at the medical school.

Some of the changes have been dictated by financial realities. Like Hebrew University, Tel Aviv University has been operating on a deficit budget, now estimated at \$7 million per year. Although its



Tel Aviv's medical school attracts students from around the world.

total deficit of around \$30 million is considerably smaller than Hebrew University's, Jacob Ziv, chairman of the Planning and Grants Committee (PGC) says Tel Aviv's problems may be greater because it is newer and doesn't have Hebrew University's large endowment. Ziv believes Tel Aviv's problems came about because the university tried to expand too far too fast, hoping the government would help out with extra money.

Life sciences have been the most successful faculty in attracting outside money at Tel Aviv University. Although it is relatively small, the life sciences faculty has attracted nearly \$4 million in outside money, approximately one third more than the medical school and the exact

sciences faculty. Physicists, however, are hurting. David Horn, chairman of the school of physics and astronomy, says the physics faculty is financially strapped. Faculty members are spending an increasingly greater portion of their time overseas where they can do research and often get larger salaries, says Horn.

But Ben-Shaul has hopes that with his blue book he can put the university onto a fruitful course. He points with pride to the fact that Tel Aviv University won 10 of the 25 Allon fellowships for new faculty members from the PGC. Ben-Shaul says the university must make cuts where needed. Although the university doesn't have everything it needs, he says, "we don't need everything we have". □

The Technion

Mount Carmel's engineering gem

THE oldest university in Israel is not the Hebrew University, as many think, but Technion, the Israel Institute of Technology. Formally opened in Haifa in 1924, one year before Hebrew University, Technion has traditionally been a centre for engineering excellence in Israel. Two thirds of all practising engineers and architects in Israel received their education at Technion. Today the university has about 8,000 students; roughly one quarter are graduate students. The number of academic staff is approximately 1,200. In addition to faculties in the engineering sciences and architecture, Technion also has departments of physics, biology, chemistry and computer science, as well as a medical school.

When it opened, Technion was located in the city of Haifa. But starting in 1954, the university began moving to new space at Technion City on Mount Carmel, a move that has only been completed in the last year or two. The medical school will remain down by the coast next to the large Ramban Hospital. Across the road from the medical school is the new Rappaport

Family Institute for Research in the Medical Sciences.

Although the Israeli government has been shifting its research support more toward the applied end of the spectrum in the past decade, Technion has not escaped the financial pinch felt by all universities. Uri Shamir, vice-president for research, describes the current financial climate as "lousy." The 1986 president's report states that in the 1981-82 academic year government money accounted for 74.5 per cent of Technion's operating budget. Today, that figure is closer to 52 per cent. Technion has pushed on several fronts to try to attract fresh endowment funds. The American Technion Society has led the way, raising \$18 million in 1985, and approximately \$25 million in 1986. Technion's goal is to double its endowment by 1990. Technion has also retained a Washington public relations firm to help smooth the grant application process in the United States.

Antiquated laboratory equipment is one of the biggest problems according to Shamir, although the university has been



Rappaport Institute's new home in Haifa.

successful in obtaining microcomputers for student and faculty use. Recruiting new faculty is also now extremely difficult because of the money shortage. Shamir says Technion just cannot offer the financial incentives to woo the most sought-after scientists, although he admits industry will always offer the more lucrative salaries. "The attraction of academia persists and will remain forever", says Shamir, "but it has fallen off some."

Electrical engineering is now one of the most popular departments at Technion. "Israel realizes now that its survival is based on the development of high technology", says professor Yehoshua Zeevi. Zeevi says Technion has "a blank check" to hire new faculty in electrical engineering, but is having trouble finding the people it wants. The shift away from basic research support is seen even in the engineering faculties. Aeronautical engineering professor Shmuel Merhav says research for its own sake has nearly vanished.

In 1982 the university established the Rappaport Institute for research in the life sciences. Director Yoram Palti says the institute has established two major research directions; the study of membranes, and an integrative approach to body systems. Palti says researchers at the institute have succeeded in developing antibodies to specific areas of the sodium channel molecule, and have begun identifying the molecular properties of those antibodies.

Work on body systems has also been fruitful. The institute has patented a new type of blood-pressure measuring device, and is pursuing research on artificial respiration using a specially designed vest. The vest vibrates at high frequencies, allowing oxygen to enter the blood from the lungs without inflating the chest.

Palti says the institute has been successful in attracting outside research money, but admits that life sciences sometimes have a difficult time winning their share of the pie in a university mostly devoted to engineering sciences. □

Hebrew University . . .

Returning to its first home

THIS has not been an easy year for Hebrew University. Last fall university president Don Patinkin resigned, and director general Israel Barghil followed suit a few months later. Revelations of financial mismanagement brought about the resignations. The specific problem was a deficit that grew from \$1 or \$2 million three years ago to between \$45 and \$80 million today, depending on whose figures are used to arrive at the total. Whatever the true size of the deficit, there is no argument that it represents a tremendous problem for the university, and its repercussions will be felt for years to come.

Hebrew University formally opened its doors in 1925 when A.J. Balfour (Lord Balfour) gave the inaugural speech. The university began with programmes in chemistry, microbiology and Jewish studies. The first campus was located on Mount Scopus, to the north of the Old City of Jerusalem. By 1947 the university had added faculties in humanities, science, education and agriculture (in Rehovot). It also became the home of the Jewish National and University Library. But the war of independence cut off the Mount Scopus campus from Israel, as the land became part of Jordan. After moving to temporary quarters scattered throughout the city, in 1953 work began on a new campus at Givat Ram near the centre of the city. Also in the 1950s construction began on a medical school campus at Ein Kerem.

The Six-Day War in 1967 restored the Mount Scopus area to Israeli control, and the university moved swiftly to rebuild its former home. By 1981 Mount Scopus was once again Hebrew University's main campus. But the cost of maintaining four separate campuses has contributed to the university's current financial woes.

Each of the three Jerusalem campuses has a different feeling to it. Approaching the new Mount Scopus campus one passes

housing developments, government buildings and a soon-to-open luxury hotel, all built since the unification of the city twenty years ago. From the campus one can find inspiring views of the city spreading out to the south. The Givat Ram pus, near the new Israeli Knesset and the Israel Museum is away from the bustle and crowds of downtown Jerusalem, retaining a spacious, suburban quality. The Ein Kerem campus is dominated by the huge Hadassah University Hospital.

There are some 17,000 students scattered among the four campuses, with nearly a third of these seeking graduate degrees. There are approximately 2,000 faculty members. Apart from clinical and public health research, all scientific disciplines are grouped in the faculty of science. The university is also the home of the Institute for Advanced Studies established by Aryeh Dvoretzky, and modelled after the institute in Princeton of the same name. There are about a dozen science research centres dedicated to research in a variety of scientific disciplines, from lasers to computer science to aquatic biology. The Marine Sciences Research Center, in the popular resort city of Eilat on the Red Sea, affords access to deep ocean conditions from just a few hundred metres off shore.

Although many universities have established separate faculties for life scientists, science dean Eliahu Friedman says including life sciences in the science faculty has brought its advantages. Physicists like Daniel Amit are now turning their attention to neural networks, creating the potential for collaboration with the traditionally strong neuroscience faculty. Friedman also praises work in chemistry, computer science, botany and mathematics. The geology department is also extremely good, but "they haven't found oil yet . . . I suppose that's not their fault". □

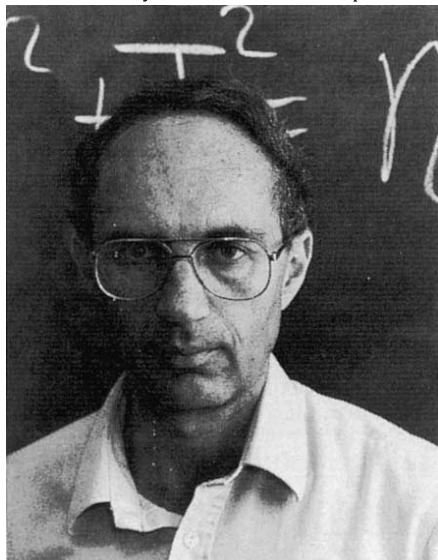


New buildings are springing up on the old Mount Scopus campus, now restored to Israel.

. . . and its troubles

With new financial woes

LIKE all universities, Hebrew University saw its share of government money shrink in the past few years. But Hebrew University has "not been able to respond to the cuts in real time", says vice-president Michael Ottolenghi. The irony is that former president Don Patinkin is one of the country's most respected economists. The university, in collaboration with the Planning and Grants Committee (PGC), is trying to work out a package that will restore financial health. Part of the plan will involve a reduction in administrative and technical staff by 20 per cent in the next five years. Faculty positions will also be reduced by between 10 and 15 per cent.



Friedman: physicists and biologists work well together in science faculty.

But a key part of the plan involves an increase of support from the PGC above the current allocation of approximately \$60 million. Whether the government will provide the PGC with the money to do this is a question that PGC chairman Jacob Ziv says will be decided in the next few weeks. A call for new elections could throw off this timetable.

Alexander Keynan, professor of microbiology and former university vice president, says half of the basic research done in the country is done at Hebrew University. The university receives nearly \$30 million per year for research. Approximately \$7 million comes from philanthropic sources, another \$12 million or so from competitive grants from within Israel, and the rest from grants from foreign countries. In 1987, the university expects to receive \$3 million of its total support from industry, but Ottolenghi says that figure will grow to \$4 million next year. That would make industry responsible for approximately 15 per cent of the university's research

budget, a figure some find troubling.

When soliciting funds for scientific research from donors in foreign countries, Hebrew University occasionally finds itself competing with the Weizmann Institute for the same money. The fear among Hebrew University scientists is that the Weizmann is better known for science, but Hebrew University is more famous for Jewish studies. The joke goes that the easiest way to raise money would be to promise to study biology of anti-semitism.

The financial strain has all but elimina-

ted money provided by the university itself for research. "I don't know how these scientists manage", says Ottolenghi. Dean of Science Friedman agrees that it is difficult to find funds for supporting junior faculty until they can find their own sources of money. But Friedman insists the university is prepared to "do whatever is necessary to keep good people."

Part of the plan to reduce faculty will also include an attempt to even out ages. Because of growth spurts in the 1950s and 1960s the faculty is dominated by senior people. □

Ben Gurion University

A desert oasis of learning

DEPENDING on how you look at it, Ben Gurion University's location in the city of Beersheva in the Negev Desert is either an asset or a liability. Because one half of Israel is desert, an academic institution concerned with growth and development of desert land seems appropriate. Thirty minutes away by car is the university's Jacob Blaustein Desert Research Institute in Sede Boqer. The Institutes for Applied Research on the Beersheva campus have many ties to the chemical industries at the nearby Dead Sea. Beersheva is unquestionably a desert environment, with camels and Bedouin camps just outside the city, but, it takes only an hour and a half to drive Tel Aviv, less to Jerusalem.

But despite the relatively short physical distance separating Ben Gurion University from Israel's main population centres, there is a psychological distance that keeps Ben Gurion University isolated. Deputy rector Joseph Pliskin says that people are reluctant to travel to the campus from Jerusalem for the day, although the same people would not think twice of making the longer drive to the Technion in Haifa.

Driving from Jerusalem to Beersheva is faster today than it was twenty years ago. It is now possible to travel a relatively straight line between the two cities through occupied territory. A picturesque if less direct route that also passes through land formerly controlled by Jordan follows the Dead Sea to the south. Travelling this way there are frequent reminders of Israeli's modern and historical security problems. Across the narrow sea, the mountains of Jordan are plainly visible. To the south, the fortress of Masada looms up, where Jews held out against the Romans in the first century, seen today as a symbol of the Israeli spirit — a kind of secular shrine and national monument.

Before turning inland, an army barricade blocks the road, suggesting that an afternoon's tranquility could easily be shattered. If the road along the Dead Sea has an arid quality, then the direct route from Beersheva to Tel Aviv quickly turns

from sand dunes to wheat fields and fertile agricultural land.

Pliskin says this psychological distance has made it hard to recruit and retain faculty. Although David Ben Gurion, Israel's first prime minister, felt that developing the Negev was crucial for the country, his hopes for Beersheva have never really materialized. Although the city has more than 100,000 inhabitants, it has few amenities besides its natural beauty and lack of traffic.

Ben Gurion is the newest university in Israel, receiving its accreditation in 1969. There are four faculties — natural sciences, technological sciences, health sciences, and humanities and social sciences — with a total student population of about 5,000. If recruitment is a problem in other areas, the medical school is an exception. A programme oriented strongly toward clinical training, it has been extremely popular among students and the object of much international interest for its approach.

School science

Giving the young an early start

IN the building next door, an accelerator is being used for nuclear physics, but in the Feinberg Graduate School the physics problems are simpler, if no less fundamental. The graduate school is the home of the science teaching department at the Weizmann Institute of Science. With a faculty of about 70, the department has been involved for nearly 20 years with developing science curricula for Israeli high schools.

Uri Ganiel, department chairman, says the Weizmann effort began with Professor

An ambitious building programme came to an abrupt halt about three years ago when the university found itself with a \$15 million deficit. For a time the situation was so bad that the university remained closed past the normal start of term until a financial survival plan could be devised. Pliskin says that plan is now in place, and the deficit has been shrinking faster than the promised \$1 million per year. Since it is so new, Ben Gurion has not had time to establish the endowments and contract ties that have helped assuage financial ills for the older, established institutions. At one point Ben Gurion was receiving approximately 80 per cent of its support from the PGC. Today Pliskin says that the figure is closer to 52 per cent, the rest coming from endowment funds (30 per cent) and direct contributions and grants (18 per cent).

The university is now anxious to resume construction to solidify the foothold it feels has been established. But thus far the Planning and Grants Committee has been disinclined to go along with such plans,



When building was in full swing, Ben Gurion University put up some spectacular structures. This is the library.

requesting that the university use any spare money to reduce its deficit. Pliskin is worried that if the government bails Hebrew and Tel Aviv Universities out of their current economic difficulties, Ben Gurion will have been "suckers" for taking steps to solve its problems on its own. □

Amos De Shalit in the late 1960s. In 1967, De Shalit together with Professor Alexandra Poljakoff-Mayber of the Hebrew University in Jerusalem had established the Israel Science Teaching Center with support from the Israeli Ministry of Education and UNESCO.

Introducing new science curricula is not easy. There is what Ganiel calls a large "spring constant" in education. "You have to be obnoxious", he says. Frequent in-service teacher training programmes are one way in which Ganiel's department

is trying to tackle this issue.

The Weizmann faculty has concentrated primarily on developing physics, mathematics and chemistry curricula. A similar department at the Hebrew University has focused on biology and senior high school mathematics, while at Tel Aviv University the effort has been on elementary school science programmes. Ganiel feels Israel has been more successful than other countries in getting new curricula into schools and revising curricula once they are in place.

Efforts to interest elementary school students in science are not confined to the classroom. Both the Weizmann and Hebrew University sponsor summer science camps with international as well as domestic participation. And on the Givat Ram campus of Hebrew University in



Professor Uri Ganiel with the nuts and bolts of his trade.

Jerusalem there is the Open Eye Center, a museum which boasts hands-on science exhibits.

University students also help with the education of their younger counterparts through a programme called Perach, the Hebrew word meaning flower. In the Perach programme, a university student will tutor one younger student two or three times per week. Once entirely a voluntary project, now students who tutor at least four hours per week can qualify for scholarships covering a portion of their tuition. The Perach programme is supported by the Ministry of Education and Culture, the Planning and Grants Committee and the National Union of Students. The programme has grown from 880 tutors in 1976 to more than 13,000 in 1986.

Ganiel says there is just now a boom in interest in physics in Israeli schools. But this popularity has underscored what is becoming a growing problem — a shortage of physics teachers. It also highlights the shortage of laboratory equipment, a problem making itself felt at all levels of Israeli education

Defence

Defence research still sacrosanct

ALTHOUGH money for basic research in Israel is in short supply these days, one rarely hears the suggestion that perhaps less could be spent on defence research. This is probably not surprising for a small country not on particularly good terms with its neighbours. Nevertheless, the latest available statistics show that military research and development accounts for nearly 60 per cent of all government money spent on research of any kind.

As in other ministries, there is also a chief scientist who coordinates the ministry's research efforts. RAFAEL, the armament development authority, and Israel Military Industries, both wholly owned by the government, together have some 20,000 employees, 10–15 per cent of whom are engaged in research activities. Industry also is an important player in Israeli military research, from giants like Israel Aircraft Industry to small, high technology companies.

Although military research had been pursued since the formation of the state and before, the real push came in 1967 when French President Charles de Gaulle slapped an embargo on all arms shipments to Israel following the Six-Day War. Realizing that dependence on foreigners for military hardware was a security risk, Israel began an extensive development programme.

Benzion Nave, a former chief scientist in the defence ministry, says the period from 1967 to 1980 saw the blooming of Israeli military research and development. Not only were new weapons developed in the laboratory, but the Yom Kippur War and later the invasion of Lebanon allowed testing under battlefield conditions. Since 1980, Nave says Israel's military research efforts have entered a more mature phase, although they have

continued to grow. In 1978/79, defence research accounted for 40.7 per cent of all government spending on research; by 1984/85 that figure had reached 58.4 per cent.

Israeli military hardware is popular in countries where it is politically acceptable to buy it. Israel's annual military hardware sales — estimated at \$1,200 million — do not come about "because of our pretty blue eyes", says Nave. Military exports account for nearly one quarter of all Israeli exports. Strengthening those exports is seen as important to help the growth of the economy.

Defence money can be important to universities. Technion has "a fair amount" of defence-oriented equipment according to vice-president Uri Shamir, although he declined to give a specific amount. In many countries the academic world is suspicious of defence-related research, but Nave explains that far from having any stigma attached to it, performing defence research is regarded quite favourably in Israel.

Defence research has also given a boost to the high-technology industry in Israel. Yigal Erlich, chief scientist for the ministry of industry and trade, says he works closely with his counterpart in the defence ministry to find new projects that will both serve a military need and be suitable for export. Some companies have been quite successful in finding commercial applications for technologies originally developed for the military.

Uzia Galil, chairman of Elron Electronics Ltd, says using technology developed for defence must be used to increase Israel's exports. Elron, a major defence contractor through its Elbit subsidiary, has sales of \$360 million, but exports made up \$220 million of that total.

Taking a share of the SDI bounty

In May, 1986, Israel became the third US ally to sign a memorandum of understanding on participation in research for the Strategic Defense Initiative (SDI) — or "star wars". So far, contracts to Israeli companies have amounted to some \$10.8 million, but that figure is expected to grow. By comparison, the United Kingdom receives \$28.9 million for SDI research, West Germany \$48.2 million, and Italy \$2.3 million. Industrial efforts in Israel will focus on electrical and chemical propulsion and theatre defence architectures.

There is only one university-based SDI project in Israel, although there were approximately one hundred projects proposed.

Financed by the Innovative Science and

Technology Office, the non-classified work is being done at Ben Gurion University in Beersheva by Professor Zamik Rosenwaks. Rosenwaks has been working on chemical lasers for the past 20 years. Since 1974 he has been developing a chemical laser that will emit at wavelengths less than one micrometre. Although chemical lasers have been successfully produced in the infrared, shorter wavelengths have proved difficult.

Rosenwaks thinks he may have found a way around some of the problems by using a pre-mixed fuel. Although he is encouraged by some preliminary results, he is quick to point out that other research has looked equally promising in the early stages, but so far no one has been successful.

Aviation industry

New plane fighting for its life

AS PART of this year's 39th independence day celebration, the Israel Aircraft Industries showed off its latest creations; two prototypes of the new Lavi fighter aircraft. The prototypes can fly, and they did so on Independence Day. Full-scale production is scheduled for the early 1990s, but whether the Lavi will ever reach that stage is still up in the air.

Lavi represents a step into the big leagues of military hardware development. Begun in 1980, the Lavi is a state-of-the-art fighter aircraft, comparable to the US-made F-16, which is already in service with the Israeli Air Force. Although nearly half of the contractors involved in the Lavi programme are foreign — mostly from the United States — the Lavi air-frame has been designed entirely in Israel, and will be built domestically. The power unit is supplied by the US company Pratt and Whitney.

Even its critics acknowledge that the Lavi is a brilliant technological achievement for a small country. But many — including some in the Air Force — believe that the Lavi is an economic miscalculation.

With a pricetag of between \$15 and \$22 million per plane, the Lavi will cost millions more than the F-16. The international political climate makes it hard for Israel to count on foreign sales to help offset production costs. Lavi has gobbled up a large proportion of available defence research and development funds, some say to the detriment of less ambitious but potentially more important projects.

The Israeli cabinet has recently been taking another close look at the advantages and disadvantages of full-scale pro-

duction of the Lavi. There has been concern in government circles about the rapid rise in Lavi's estimated production costs. The pricetag has prompted rumblings from within the armed forces that perhaps there might be better ways of spending the defence budget. Although the Lavi will have performance characteristics that enemy forces will not be familiar with, it is unclear just how useful an advantage that would be.

But cancelling Lavi would have economic repercussions. The market would be flooded with unemployed aeronautical engineers, many of whom would no doubt leave the country. In addition, such a major project involves more than just the aircraft industry.

Full development would be a huge push to technology in Israel, says former defence chief scientist Benzion Nave. Supporters also reject the idea that cancelling Lavi would free up money for other projects. Shmuel Merhav, professor of aeronautical engineering at the Technion, says the short-term financial reward for those pinched by Lavi development would be more than offset by the damage to interest and leadership in the field. Without Lavi, Merhav argues, the nucleus of the Israeli aircraft industry would be damaged, and it would be hard to attract the next generation of students to replace it.

Israeli industrialist Uzia Galil agrees that Lavi is "an expensive game for a small country". But Galil believes the question has now become not whether the Lavi will go into production, but how many will be built. While politicians are arguing, Galil says, industry continues to prepare the Lavi. □

Communications

Staying in touch from afar

ONE of the greatest successes Israeli scientists have managed is staying plugged in to the international science scene despite being geographically isolated. Part of the success comes from the traditional practice of sending every graduate student abroad for postdoctoral training. Those that return have usually formed close associations with laboratories and scientists overseas, contacts which often lead to continuing collaboration.

For more senior scientists, an annual travel allowance makes at least one trip out of Israel a year fairly easy. There is also a flurry of international scientific meetings in Israel each year, and visiting faculty are welcomed. Israeli scientists are hungry for news from abroad, and in the opinion of one senior scientist who asked not to be named, Israelis are often more up to date than their colleagues at many US universities. Michael Sela, former president of the Weizmann Institute, likes to brag that there is "not one piece of relevant scientific gossip that doesn't reach us in 24 hours."

But the BITNET computer network has really given researchers in Israel an inexpensive way of keeping in touch. In the summer of 1984, Israel became only the second country to link into the main European node in Pisa, Italy. IBM has underwritten the cost of BITNET since its inception, but that arrangement ends at the end of this year. Joe van Zwaren, currently the coordinator for physics in the ministry of science, sold the BITNET concept to many skeptical scientists now addicted to it. He is concerned that paying for the network may be difficult when IBM pulls out.

Most people use BITNET as an electronic mail service, sending letters and manuscripts to colleagues in other countries, although it can also be used to send raw data files. Physicists were the first to use the system, but biologists are now also becoming hooked. There are now 5,000 users in Israel, more than one quarter of the teaching staff of all the universities. van Zwaren is now using BITNET as a bulletin board, publishing frequent newsletters on currently hot topics such as superconductivity.

The mail service is notoriously poor in Israel, and the telephone system is not much better. BITNET is "a mode of communications that makes you feel close to where the action is," says David Horn, professor of physics at Tel Aviv University. Adds biologist David Mirelman of Weizmann, "this is really making a revolution for us." □



The pride of the Israeli aircraft industry. But it's expensive.

Science-based industry

Where the government is aiming

THE key to success for science-based industry in Israel, says chief scientist Yigal Erlich of the Ministry of Industry and Trade, is to create a market niche by anticipating a need other companies have overlooked. Erlich, with his \$80 million budget, is trying to help companies find those niches and develop products to fill them.

Science-based products have been an increasingly important part of Israel's economy. In 1970, exports in metals, machinery and electronics were \$70 million. By 1984, that figure had jumped to \$1,756 million, and will certainly continue to grow.

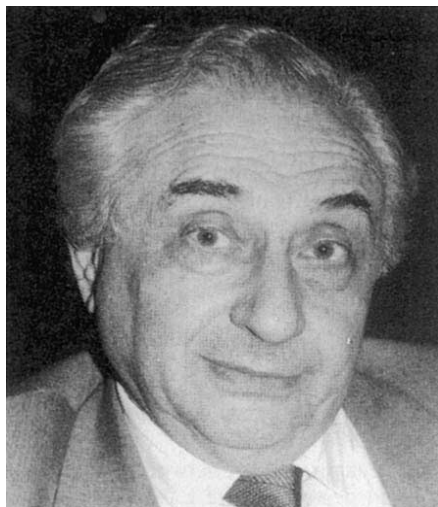
Optrotech is an example of a company that found a niche. Together with its competitor Orbot, the two companies control nearly the entire world market in automated inspection systems for printed cir-

cuit boards. The systems use specially designed hardware and software based on artificial intelligence research to scan commercially produced printed circuits for defects. In the first six months of 1986, Optrotech's revenue was more than \$12 million, compared with total annual sales of \$3 million just three years earlier.

Optrotech is one of the successful subsidiaries of Elron Electronics Industries Ltd, a company founded by Uzia Galil. Galil got his industrial electronics experience in the 1950s when working for Motorola in the United States on the development of colour television. He returned to Israel after leaving Motorola, and joined the faculty of the Technion in Haifa. Galil says that, at the time, Israeli science was ruled by the academic com-

munity, but his experience in the United States had shown that academic science could be a powerful force for industry.

Starting with \$160,000 in capital in 1962, Galil began to look for products to develop in fields where Israeli research was already well established. Today Elron is a holding company for some six high-technology companies with aggregate sales of \$360 million. Elbit, Elron's major subsidiary, designs and manufactures computer-based electronic systems for defence and industry. Another subsidiary, Fibronics, builds fibre-optic communication systems. Other subsidiaries are in



Galil — combining science and industry.
digital signal processing and computer integrated manufacturing.

Biotechnology a favoured child

THE promise of biotechnology has attracted interest in Israel just as it has in most countries. But Israel has advantages to set it apart from the "me-too" players in the biotechnology game. The immobilized enzyme system used in bioreactors and bioanalysers was developed by an Israeli scientist, Ephraim Katchalski-Katzir, and other scientists from Israel have done seminal work on synthetic peptides, synthetic antigens and, more recently, growth factors. But even with these advantages, turning a profit in biotechnology will not be easy, as

provided the Israeli company with the necessary international marketing force, but Bio-Yeda had to make its own arrangements. Since the Israeli market for biotechnology products is small, international distribution is crucial. After struggling with this issue for three years, Yeda sold the company to Makor Chemicals, a subsidiary of the US chemical company Sigma, with a name change to BioMakor.

Charles Hexter, deputy managing director at BioMakor, says that the company works at a "basically scaled-up laboratory size". Research immunochemicals were the first products, and remain BioMakor's main focus. The company's annual sales are about \$3 million, 90 per cent from exports. Hexter says scientific talent in Israel is as good as anywhere, but market feedback is a problem. He believes it would "very difficult if not impossible" for an Israeli company to penetrate an international market without a US or European partner.

Despite the difficulties, more biotechnology companies are springing up in Israel, and biotechnology is frequently mentioned as a growth area for Israeli industry. Bio-Technology General and InterPharm Laboratories are, respectively, conducting clinical trials of human superoxide dismutase and developing a recombinant beta interferon.

Katchalski-Katzir, head of a recently formed biotechnology department at Tel Aviv University, believes the government must provide more money during the difficult intermediate stage between product development and marketing. But Hexter says expectations should not be too high. Even if the biotechnology industry thrives in Israel, its scale will be insignificant compared with the United States. □



Hexter — penetrating the market.

older and better-situated companies have proved so effectively.

The first biotechnology company in Israel was Miles-Yeda. Founded in 1967, Miles-Yeda was a joint venture between the large pharmaceutical company Miles, and the commercial holding company for the Weizmann Institute, Yeda. But after the West German company Bayer purchased Miles, Miles sold its share to Yeda, and the company became known as Bio-Yeda in 1983. Miles' exit caused a stir, as some suggested it was prompted by an Arab boycott of Bayer products, a boycott that was lifted following the sale.

The arrangement with Miles had pro-

But one Elron company, Elscint, has fallen on hard times. A manufacturer of computerized tomography and magnetic resonance imaging systems, Elscint expanded too far too fast, and ran up losses estimated at about \$150 million. Galil says the company is now back on its feet but Elscint's problems scared Israel's high-technology industry.

Scitex, another of the stars of Israel's science based industry has also recently fallen on hard times. Scitex manufactures interactive computer graphics systems that are used throughout the publishing world. Up to the third quarter last year, Scitex had lost \$14 million on revenues of nearly \$86 million. Neither Scitex nor Elscint consider that their technology is to blame for their recent financial problems.

Erlich is trying to enlist universities' talent in the search for new market niches. He reckons 10 per cent of his budget is going to universities for cooperative centers with industry.

Erlich's office now supports projects at some 400 companies. He sees himself more as a "chief venture capitalist than a chief scientist". The ministry receives a 2 per cent royalty from projects it has supported, and revenue is now about \$5 million a year. Erlich expects his budget will continue to grow, as Israel continues to push for high-technology triumphs. □

Tropical medicine

Coming to grips with invaders

PARASITIC diseases, although not a major problem in Israel, are nonetheless a significant health hazard in the Middle East, especially in developing countries. But collaborations with Egyptian scientists, the unfamiliar health problems of the Ethiopian Jews who emigrated to Israel in 1983 and the interest of a US foundation have all conspired to promote research activity in parasitic diseases.

At the Kuvim Centre for the Study of Infectious and Tropical Diseases, professor Charles Greenblatt has been studying leishmania, a parasite that causes leishmaniasis in humans. Infection generally causes only disfiguring skin lesions, but in severe forms it can be fatal. Following the Camp David agreements, health exchanges began with Egyptian scientists, supported by the US Agency for International Development.

Although collaborations cooled in 1982 following Israel's invasion of Lebanon, a serious form of leishmaniasis broke out near Alexandria, and Greenblatt travelled to Aswan to help with control measures.

Dan Spira, who heads the malaria unit at the Kuvim Centre, helped to organize an extremely effective campaign against that disease when it appeared in Israel with the arrival of the Ethiopian Jews. Spira and his colleagues assisted the health ministry establish treatment protocols and preventive measures. The vector for malaria, the anopheles mosquito, exists in Israel, making a rapid response all the more important. Since 1982 there has not been a single case of malaria in the country.

At the McArthur Center for Tropical and Parasitic Diseases at the Weizmann Institute, director David Mirelman is also interested in the health problems of Ethiopian immigrants. The centre, now supported by the McArthur Foundation but initially funded under the Rockefeller Foundation's programme for Great Neglected Diseases, has been studying the molecular basis for pathogenicity of amoebae. Although there are hundreds of millions of people worldwide who carry parasites, only 10 per cent ever show evidence of disease. This has led to the view that there are two morphologically identical forms of amoeba, one pathogenic and the other not.

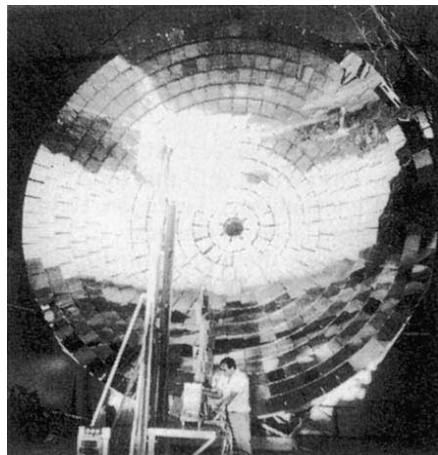
But Mirelman now believes that there may be a different explanation. It is possible that an amoeba may differentiate after invading its host, so that the same amoeba may behave differently in two different hosts. The question then is what causes this differentiation. Mirelman believes a parasite's pathogenicity may be determined by the bacterial population in an individual's digestive tract.

It is possible that individuals with AIDS (acquired immune deficiency syndrome) may be particularly susceptible to disease from parasitic infections. Because homosexual populations can transmit parasites with relative ease, Mirelman thinks it is important to develop less toxic treatments

for parasitic infection, to drive them out before they turn into pathogens. Ultimately, however, Mirelman believes vaccines will be necessary to control parasitic diseases, especially in developing countries where there are repeated exposures. To this end, he and others at the McArthur Center have been studying the lectins that permit amoebae to attach to cells. Interrupting lectin production may be a route to blocking infection. □

Not much oil, but plenty of sunlight

ENERGY self-sufficiency is a distant and probably unattainable goal for Israel. At the start of this decade, oil supplied 98 per cent of Israel's energy needs, and 99 per cent of that oil was imported. Today, with the completion of a new power station in Hadera, coal supplies 55 per cent of the electricity and 17 per cent of the overall needs. With the addition of another coal-



Weizmann's solar furnace can produce temperatures of 3,000°C.

fired plant in Ashqelon those numbers will change to 70 and 27 per cent. But the reality remains that unless the world economy changes dramatically, fossil fuels will be the main source of Israel's energy needs.

Israel has had an historical interest in solar energy. In 1954 Harry Z. Tabor began a project to develop selective surfaces to improve solar-collector efficiency. Solar collectors are a common site today on homes throughout Israel. Technology for generating electricity from solar ponds has been developed by Luz International, a US company with an Israeli subsidiary, and a 5-megawatt plant at Bet HaAravah is now on the national electricity grid.

The newest effort in solar energy research is the Central Receiver facility just being completed at the Weizmann Institute. With a \$10 million price tag and cost overruns, the new facility has been unpopular among some of the faculty at Weizmann. But the new facility will provide the means to test new ways of using Israel's abundant sunlight.

At noon, 64 mirrors can concentrate 3,000 kilowatts of power on one of three different experimental stations built into the solar receiver. Israel Dostrovsky is director of the new solar facility. Since only one-third of Israel's energy consumption is in the form of electricity, Dostrovsky and his colleagues have been interested in other ways to use solar energy besides generating electricity.

Dostrovsky is particularly interested in the chemistry of reforming reactions. For example, methane and carbon dioxide converted to hydrogen and carbon monoxide. That mixture can be transported or used as a source of new chemicals or fuel. The new facility will be used to test solar powered lasers, as well as for studying new methods of direct and indirect solar heating. Many of the projects planned for the solar tower have been tested on a smaller scale in the solar furnace already in operation.

Apart from nuclear energy research — controlled by the Israel Atomic Energy Commission — the Ministry of Energy and Infrastructure directs energy research activities. In the past, solar energy research received about half the ministry's research budget.

For 1986, the total research and development budget was \$6.7 million, down from a peak of \$15.5 million in 1982. Oil shale research received the largest share of last year's research budget (50.9 per cent), with solar research receiving slightly less than a quarter of the 1986 total.

Beginning in 1984, the energy ministry made a concerted effort to shift its research budget toward support for projects that would show a commercial return in 10 years. Many projects that had been technical successes were not economically viable because oil prices failed to climb as fast as expected. One ambitious project, now set aside, was to divert water from the Mediterranean to the Dead Sea.

Nuclear energy is not considered likely to be part of Israel's energy future. There is a 5-megawatt pool type plant at the Soreq Nuclear Research Plant near Tel Aviv, and a 25-megawatt natural uranium fuelled reactor at the Nuclear Research Center - Negev, in Dimona. Some consider Israel's interest in nuclear reactors to be part of its efforts to construct a bomb. □

Agricultural research

Farmers and scientists combine

AGRICULTURE in Israel is a modern success story. Despite being a country that is half desert and suffers from a chronic shortage of water, Israel has now become virtually self-sufficient in agricultural products. Not only that, but agriculture now provides Israel with much needed foreign currency — exports were worth more than \$500 million in 1984.

Research in agriculture was among the first scientific activities of the new state of Israel. Even before independence, Professor Eliezer Volcani founded the Agricultural Experiment Station in 1921 in the new city of Tel Aviv. Today the station is in Bet Dagan, and it is now called the Volcani Institute of Agricultural Research, a part of the Agricultural Research Organization in the Ministry of Agriculture.

Two factors have played significant roles in the success of Israeli agriculture. First, food production was considered important for the security of the state. Sugar beet production, for instance, continued long after the country could afford to purchase sugar more cheaply from other producers because of the fear that the supply could be restricted at any time. For a country anxious to sustain economic growth, producing a steady supply of food was a must.

The other factor was philosophical. One faction of the Zionist movement that brought people to Israel advocated a "return to the land and to physical

labour". The elite of Jewish society was willing, even anxious, to leave the cities to work on the farms, both to build the state and to seek personal redemption. Ilan Sela, professor of virology at the Hebrew University's faculty of agriculture in Rehovot, says it is a combination of good science and an intense interest in improvement from the people actually doing the growing that has propelled Israeli agriculture along so rapidly. Far from being suspicious of new or experimental approaches, farmers are knocking on the doors of research stations asking for fresh ideas.

Israel's commitment to agriculture has meant relative financial health for agricultural research. The United States-Israel Binational Agricultural Research and Development Fund (BARD) provides approximately \$8 million per year for joint projects, and West German funding agencies have also been willing to invest in Israeli agricultural efforts. The German Minerva fund provided DM 1 million for the new Otto Warburg Centre for Agricultural Biotechnology, based on Hebrew University's Rehovot campus.

But there are those who worry that agricultural research is losing some of its appeal. Professor Uzi Kafkafi of Hebrew University says that bright students who might once have been attracted to agriculture are now turning to fields such as computer science, drawn by the promise of better support and higher salaries. □

Water supply

Water, water hardly anywhere

WATER is the limiting factor to the growth of Israeli agriculture. The Israeli water authority is familiar with the water content of nearly every square metre of the country, both above and below the surface. Israel makes use of nearly every drop of the 1,600 million cubic metres of fresh water available annually in the north. Techniques for stretching the water supply, from drip irrigation to the use of catchments for rainfall, are constantly being developed and refined.

Desalination has not proved economically viable, and there is now great interest in growing crops in water with relatively high salt concentration.

Hebrew University's Professor Uzi Kafkafi has been studying the genetic aspects of plant resistance to salt. Kafkafi says plant salt sensitivity was once considered to be simply an osmotic problem, but this is now thought unlikely, since he and others have developed plant strains within a species that have different growth rates when grown at the same salt concentration.

At the Ben Gurion University Desert Research Institute in Sede Boqer Professor S. Herman Lips is also interested in the genetic properties of halophytes — plants that can grow in salty soil. He is looking for the specific proteins in halophytes that give them their salt resistance. Also in Sede Boqer, Moshe Silberbush is studying ways of using the ample quantities of brackish water in desert aquifers to grow cash crops. Drip irrigation slows evaporation that concentrates salt, and permits the use of water that is at the upper limit of salt concentration for salt-sensitive plants. Fertilization management may extend that limit further.

But even if the problems of high salinity are overcome, growing in desert sand presents another spate of problems. Low water-holding capacity, low levels of nutrients and micronutrients, and reduced local water flow all present difficulties for crops. But as researchers in Sede Boqer are quick to point out — and indeed it is impossible to forget — there is plenty of sunlight and warmth.

Still, given increased supplies of water, Israeli researchers remain confident that the Negev and the nearby Sinai could be made into some of the greatest growing regions in the world. The water exists, but it is flowing in the Nile some 200 kilometres away, a long way geographically but even further socially and politically. Says Kafkafi rather wistfully, "I wish we had one per cent of the Nile." □

Coaxing trees to produce longer fibre cells means that each tree yields more pulp for paper. Dr Roni Aloni of Tel Aviv University has shown that treating young trees with the plant hormones auxin and gibberelic acid brings dramatic results — the treated plants are on the right. Fibre growth can double in about three months.



Farmers acquire a taste for success

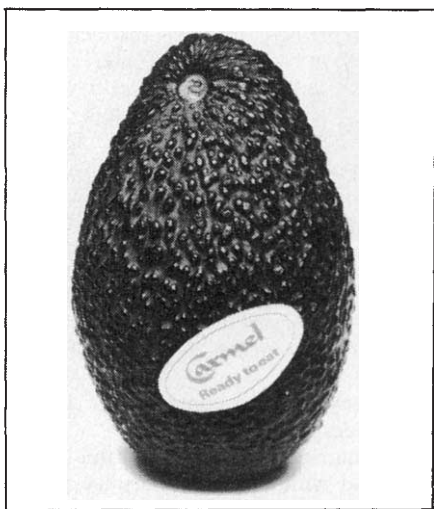
FARMERS in Greece and Spain have distinct advantages over their counterparts in Israel. Apart from the relatively greater abundance of water, those countries have much shorter distances to ship their produce to market. To hold on to its market share in Europe, Israeli farmers feel they must find an edge that will help overcome their logistical disadvantages.

There are numerous examples of market successes:

- **Tomatoes.** Once considered "a four letter word" because of a growing programme that resulted in tomatoes that rotted before they could reach their market, tomatoes are now a success story. Nachum Kedar of Hebrew University has succeeded in breeding a tomato that can be picked when it is very nearly ripe — with its full flavour — and still have a two-week shelf life, long enough to reach the growing European markets.

- **Avocados.** Brought to Israel from Mexico, there is a market for them in Europe that never existed before the Israelis started supplying it.

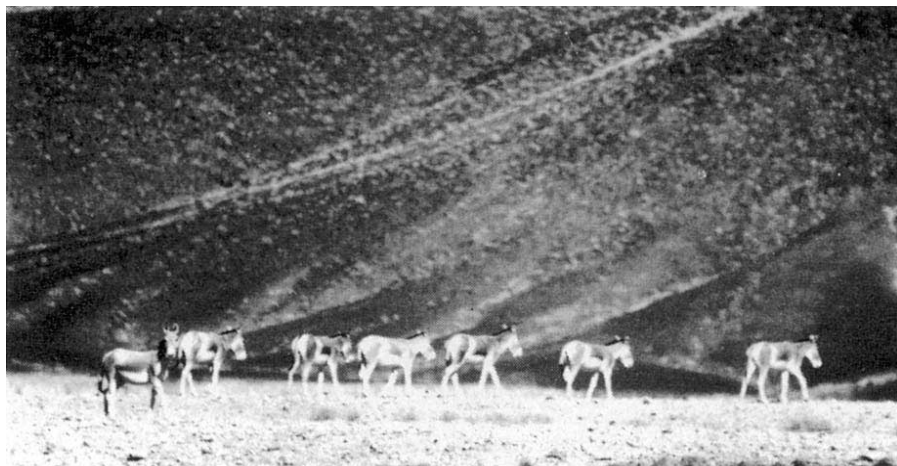
- **Goose liver.** Israel is now providing a sizable proportion of the livers used for making paté de foie gras in France.



The Hass variety of avocado — making inroads into European cuisine.

Nachum Snapir at Hebrew University is working on a method of using stereotaxic surgery to destroy a goose's ventromedial hypothalamus. This results in a bird that will regulate its body weight at nearly twice normal, yielding fat livers with no further intervention.

- **Mushrooms.** Researchers in Rehovot have found that the edible oyster mushroom will grow on cotton straw. This provides the double benefit of a cash crop and of a straw that can be used for livestock after the fungus breaks down some of its lignin. □



Once a familiar sight, now it takes sharp eyes to spot the "swift ass" of the Negev. See p. 584.

The Negev

Science takes root in the desert

SOMETHING about the the Negev desert, and Sede Boqer in particular, seems to symbolize the very existence of Israel.

Certainly Israel's first prime minister David Ben-Gurion felt a special affinity for Sede Boqer, for he moved there in 1953 at the age of 67, and both he and his wife are buried there. Perhaps the appeal comes from the challenge.

At the Jacob Blaustein Institute for Desert Research — a part of the Ben Gurion University — the goal is to make the desert more habitable. To this end, there are research efforts to squeeze more water from sparse rain clouds, introduce new crops that will thrive in the abundance of heat and sunlight, and figure out ways to make use of the brackish water that lies in underground aquifers.

One recent success is a special greenhouse that takes advantage of desert conditions. Moshe Zeroni is acting director of the controlled environment desert agriculture unit at Sede Boqer. In this greenhouse, plants only need about 10 per cent of the normal water requirements, because water is not lost in the enclosed environment. The problem is that they do poorly when water condenses on their leaves, a problem in the high-humidity greenhouses.

Moreover, any greenhouse that lets in enough light also heats up very quickly in the desert. Zeroni and his colleagues think they have found a clever way around these problems. They have constructed a double-walled greenhouse through which a liquid filter passes. The filter blocks 94 per cent of the infrared sunlight, but transmits 85 per cent of the photosynthetically active radiation. The filter then passes to a heat exchanger that heats a water tank. The cooled filter returns to the walls of the greenhouse, and because it is cooler than the interior of the greenhouse, water con-

denses on the walls of the greenhouse rather than on the plants.

Zeroni says even in a high humidity, plants can thrive so long as water does not condense on the leaves. The process reverses at night, when the heated water is used to raise the temperature of the filter passing through the greenhouse walls, insulating the plants from the cold desert nights.

Ironically, the most likely cash crop from the greenhouse appears to be house plants. Zeroni is now working on a simpler version of the greenhouse for export.

Just past the greenhouse, Amos Richmond is coaxing algae to grow in specially constructed ponds. Many species of algae like the high solar radiance and warm temperature of the desert. The trick here is to generate the right amount of turbulence in the mass of growing algae so that each alga gets its moment in the sun.

So far, Richmond has only been getting photosynthetic efficiency around 1 per cent, and he says that 5 per cent efficiency is needed to make algae growing commercially viable. In the laboratory, Richmond has achieved efficiencies as high as 7–8 per cent. The dried algae can be made into an edible if somewhat strong-smelling substance. The hope is to make algae the "manna of the desert", says Richmond, but so far "instead of making a cheap source of protein for the very poor, we are producing expensive health food for the very rich."

The desert may seem an unlikely place for fish farming, but that is also being tried at Sede Boqer. The warm, brackish water in desert aquifers apparently is quite acceptable to eels, and a pilot project is under way to see if they can be grown commercially. Ironically, eels are not kosher, so the gustatory success of the project will be determined by reviewers elsewhere. □

Desert conservation

Onagers return to the Negev

"Who hath let the wild asses go free? Who hath loosened the bonds of the swift ass?" (Job 39:5). These days, it is the government-financed Nature Reserve Authority (NRA). Last month, a group of onagers, the wild asses of the region, were released into Israel's southern Negev desert, part of an attempt to show that this was once the onager's original habitat.

Since 1981 the NRA has had mixed results with its reintroduction plans. Four groups of onagers have been set free in the Ramon Crater, a 400 km² wilderness. The asses made their way to watering holes — natural and artificial — in the steppes region of the desert and near the Jordanian border, but days before the fourth group was due to be released last month, two males of the group of eight broke through their enclosures and the others were allowed to follow. One of the mares was fitted with a transmitter, but she is now lost. The rest have been sighted.

Archaeological digs in Israel have turned up the remains of *Equus hemionus onager*, as well as the African and Syrian subspecies of wild ass. Only 300–400 of the asses remain in the wild today, mostly in the plains of Iran. The onager is more dependent on water than the African wild ass, and its disappearance from Israel may be linked to Bedouins and their domestic herds encroaching on its water resources.

It is an ironic twist to the age-old efforts to tame them that Israeli authorities are trying to bring these "fearless" creatures back. After having disappeared for a half century, three pairs of onagers were imported from Iran in 1968. These were kept in captivity in the 12 km² Hai-Bar Biblical Wildlife Reserve, one of the latter-day Noah's Arks in Israel that are attempting to revive and protect wildlife as it was in biblical times. Among the many creatures that have come under the reserve's wing are the Mesopotamian fallow deer, the Cyprian wild sheep, ostriches and addaxes. Four pairs of white oryxes arrived at the reserve from Saudi Arabia, after first being shipped to the United States. The Saudi authorities were unaware of the animals' final destination.

"The Negev needs large herbivores to balance the desert ecosystem," says Avinoam Luria, head of the NRA wildlife division. Reintroduction of the onagers is thus partly to bring a biblical flavour back to Israeli landscapes, and partly as a food source for griffons and lappet-faced vultures, predators also threatened with extinction.

Lisa Perlman

Lisa Perlman is on the *Jerusalem Post* staff.

Ministry of science

Cabinet newcomer enters fray

THE Ministry of Science and Development, formed in 1982, did not come about because of a perceived need for a coordinated science policy. Instead, it was part of a political deal with a small right-wing political party called Techiya — the Hebrew word for renaissance. In return for its support for the ruling Likud party, Techiya was rewarded with a seat in the cabinet.

But if the deal was political, Techiya had a man for the post whose scientific credentials were beyond reproach. Knesset member Yuval Ne'eman, a particle physicist on the faculty at Tel Aviv University, is frequently mentioned as an Israeli scientist who could well win Israel's first Nobel prize in science. Ne'eman was science minister from July 1982 until September 1984, when the new coalition came to power. Should early elections be called, Ne'eman believes there is a chance that he will once again return to the cabinet.

Israel made a commitment to a science policy body from the earliest days of its existence. During a cease-fire in the 1948 war of independence, David Ben-Gurion's government created the Science Council, a body that later became the National Council for Research and Development (NCRD), responsible to the prime minister until the creation of the science ministry and ostensibly in charge of all government science. With the creation of the offices of the chief scientist in the different ministries, the government's scientific efforts were necessarily decentralized.

Gershon Metzger, deputy director general of the science ministry, says that the ministry is mainly a coordinating body, keeping track of what is going on in Israeli science and suggesting new directions. The ministry also oversees the Israel Space Agency. Metzger says his office maintains a database with information on all science projects throughout the entire country.

Coordinating scientific exchanges is a large part of the ministry's efforts. In addition to the three United States–Israel binational funds (see page 570), a new binational fund has been established with West Germany. Each country contributed half of the fund's endowment of DM 150 million. The first grants will be awarded at the end of this year.

Without a strong force as minister, Ne'eman feels that the science ministry has lost some of its recently gained

momentum. The current minister in office, Gideon Patt, is a member of Likud but is not a scientist.

Ne'eman counts among his successes as minister the doubling of the endowments for the three binational funds with the United States, an achievement he credits to his close relationship with the former White House Science Advisor George Keyworth.

That relationship was probably also helpful in steering research money for the strategic defense initiative (SDI) Israel's way. Ne'eman also established the Yiftach commission to analyse Israeli basic science. Among its recommendations, the Yiftach commission recommended that a central body be placed in charge of basic science policy, and that a science foundation be created to support research. Although the report's recommendations have been accepted by the cabinet, there has as yet been no specific action taken to implement them.

One of the recommendations that Ne'eman feels strongest about is moving the budget for higher education out of the education ministry, which, he says, "puts the universities in a difficult situation



Ne'eman (left) feels that Israel missed an opportunity in solar energy. Gershon Metzger (right) is charged with keeping track of research in progress.

when they have to compete [for funds] with kindergartens".

Ne'eman is afraid Israel's timing has been bad with respect to science policy decisions. As an example, he cites Israel's early advantage in solar energy. By the 1960s, Israel had begun to develop cost-effective methods of using solar energy. But by failing to support such basic energy research in the face of declining oil prices, Israel found itself in the same boat as all other Western nations when oil prices skyrocketed.

Shalom Abarbanel is the current chairman of NCRD. An applied mathematician at Tel Aviv University, Abarbanel also believes in the necessity for strategic planning in Israel's science policy. "It is not clear", he says, "that the government understands in its gut the importance of basic research to health of the country." □

Jewish law

Science and the Jewish tradition

THE current Jewish year, 5747, is a sabbatical, not in the academic, but in the original sense of the word—the seventh year in which fields must be left unploughed and vines unpruned. This prescription, a valuable, but no longer valid early contribution to conservation policy, has been traditionally circumvented by symbolically selling the land on the eve of the sabbatical to a friendly Gentile, then “purchasing” it back as soon as the sabbatical is over. But a recent upsurge of fundamentalist “ultra-orthodoxy” in Israel has this year produced complications.

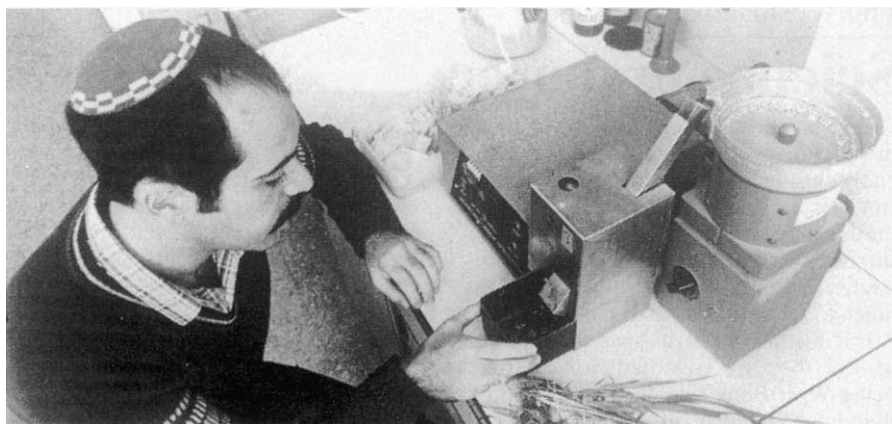
The politically potent ultra-orthodox object to wheat grown within the state of Israel being sold to flour mills and bakers. This could mean that the entire crop will have to be written off, with a massive subsidy to the farmers, although the government is trying to work out a compromise by which a limited amount of foreign wheat will be imported for use by bakers patronized by the ultra-orthodox.

The present controversy over the wheat highlights a problem which has faced the state of Israel from its inception 39 years ago. Although not technically a theocracy, state structures conform to the tenets of orthodox Judaism. In addition the state attempts to ensure that its citizens, whatever their degree of observance, have maximum facilities for fulfilling their religious duties as they see them.

This can lead to major confrontations, as when archaeologists, about to excavate a site, face the Talmudic prohibition on disturbing graves. More often the consequences are more amicable. Dr Rose Bilbul, a scientific entrepreneur who combines the commercial growing of papayas with research on the properties of papain, wished to sell her produce to a leading hotel. But the papaya, in spite of its appearance, is not, botanically speaking, a tree. The hotel insisted that expert advice be taken to assure that the appropriate blessing could be pronounced on the grove so that the hotel would be sure of maintaining its kosher kitchen.

Judaic law is essentially fixed, so scientific innovations pose challenging problems. Jewish law prohibiting cross-breeding and the production of chimaeras, for example, seems at first glance to rule out genetic engineering. But the rabbinic tradition has centuries of expertise in dealing with such situations, and ways have been found to permit such work to continue.

Israel has one institution specifically devoted to unravelling such problems, the Institute for the Study of Agriculture according to the Torah. The institute specializes in the development of biennial



Graduate student Avraham Levy experiments with wheat grains.

crops that can be sown before and harvested after the sabbatical year.

Orthodox restrictions have spawned some ingenious devices. The prohibition against kindling a fire on the Sabbath (which is considered by the strict Orthodox to include switching on an electrical current) prompted domestic lighting sys-

tems which respond automatically to external twilight or darkness. The Sabbath telephone answers the phone automatically, placing the caller on hold. Since the connection is already established, the recipient of the call in picking up the receiver does not perform “work” in the Talmudic sense.

Vera Rich

Immigration

A difficult transition

THE past twenty years have seen a major wave of Soviet immigrants to Israel. Though numbers fell to a few dozen per month in the pre-Gorbachev years, they never entirely dried up.

Jews make up a disproportionately large part of the Soviet scientific community. Although some Jewish scientists were temporarily or permanently refused an emigration visa on security grounds (the “refusniks”), and others simply used a visa for Israel as the first step to some other country (generally the United States), the influx of ex-Soviet scientists has been more than the Israeli scientific establishment could easily absorb. Yet the Law of Return guarantees any Jew repatriation in Israel, difficult as it might be to provide a suitable post for an incoming particle physicist or antarctic geologist.

The narrow specialization of Soviet higher education can make the transfer to some new speciality difficult. In some sense, the situation is less traumatic for a long-term refusnik, who is well aware that, in his years of waiting, he has fallen irrevocably behind with his professional reading, and who knows that his professional life in Israel must involve a new start. Resettlement is more complicated for the young engineer who arrives after a relatively short delay, only to find that his Soviet qualifications are too narrow, or simply not up to Western standards.

Over the years, various solutions have been proposed for the integration of refusnik scientists: a technology kibbutz in the Golan, endowed bridging grants for resettling, even the creation of fictitious one-man “departments” to cushion egos

still attuned to a system where status is all important. Not all can make the psychological adjustment. Certainly, it is easier to absorb, say, a mathematician, who needs only a pencil and paper and desk than, say, an organic chemist or metallurgist who needs an expensive laboratory. And those who find room in the Israeli academic establishment have to adjust to a new way of working.

Israeli politicians and activists for *aliya* (immigration to Israel) state publicly that they will absorb any number of ex-Soviet scientists “even if it means wall-to-wall academics from Dan to Beersheva”, as Golda Meir said at the Second World Conference for Soviet Jewry in 1976. But away from the rostrum, they have to face the reality that between Dan and Beersheva there are few posts for massive numbers of new researchers.

Last month, a Canadian all-party delegation visiting the Soviet Union on behalf of Soviet Jewry was given a broad hint that, if the West would cooperate with the Soviets on computer technology, the Soviet Union might lift the “security” restrictions on refusnik scientists. However much the Israelis might welcome such an agreement, there will still remain the problem of where to deploy the scientists so released. The best-known would undoubtedly be welcome elsewhere, especially in the United States. But after long years of waiting, it would be ironic if Israel could find them no appropriate job, even more ironic if those who have campaigned so long for their right to live as Jews in Israel should be obliged to take work elsewhere.

Vera Rich

The West Bank

Science under occupation

ACADEMIC science on the West Bank is just getting off the ground, and there are enormous hurdles yet to be overcome. But just as Israeli scientists feel drawn to the Jewish State, and are willing to make sacrifices to do research in their homeland, so Palestinians are willing to give up much to fulfill their own dreams.

But if Israeli scientists have at least a say in how they are governed, for the time being West Bank scientists do not enjoy that luxury. Universities can be closed without notice, wreaking havoc with experimental protocols and disrupting the fragile fabric of the new scientific enterprise.

On 13 April, violence broke out at a rally of students from Bir Zeit University in Ramallah. The students were protesting at attacks by Jewish settlers on an Arab village after a Jewish West Bank settler had been killed some days earlier.

One student was shot and killed, three others were injured by Israeli soldiers. The next day, military authorities ordered the university closed for four months.

"It is awful when the university closes", says Ali Fattom, a member of the biology department at Bir Zeit, now on a postdoctoral fellowship at the National Institutes of Health in Bethesda.

But closures have been nearly an annual occurrence. Animals must be abandoned, experiments stopped and offices closed, until the campus reopens. Fattom once had to leave his laboratory with live streptococcus on a laboratory bench.

Abdul-Ghani Abdul-Salam is also on the biology faculty at Bir Zeit. Born in Acre near Haifa, Abdul-Ghani holds an Israeli passport, and received his PhD in neurochemistry from Hebrew University Hadassah Medical Center in Jerusalem. He held a postdoctoral fellowship at Imperial College in London, where he worked on the neurochemistry of epilepsy. But when his first child was born, he chose to return home, joining an Arab rather than a Jewish university.

Although Bir Zeit has approximately 50 people in its science faculty, research activities at West Bank universities are just getting started. Abdul-Ghani had to start from scratch in establishing his research laboratory at Bir Zeit. He has been developing a chronic model of epilepsy in rats, and has published preliminary results in the *Journal of Neurochemistry*. When the university closed, all his animals were lost, making him both angry and frus-

trated. "What do my rats have to do with the security of Israel?", he asks.

Ghassan Yassim, dean of science at Bir Zeit, says West Bank universities must be politically active, even if that upsets the authorities, and they must also stay in touch with their communities. But he

Scientists of the universities on the occupied West Bank understandably consider themselves not to be part of Israeli science.
J.P.

agrees that the university as a whole should not be punished when students protest off-campus.

There are approximately 2,400 students and 200 faculty at the 12-year-old university. There are four universities besides Bir Zeit on the West Bank — Abu-Dis, An-Najah, Hebron and Bethlehem. None receives support from the Israeli government, but are financed instead by local



West-Bank scientists Ghassan Yassim (right) and Abdul-Ghani have to make the best of a complicated life.

philanthropists and neighbouring governments. West Bank universities forbid formal contacts with Israeli universities, because of the political inequalities. For example, says Fattom, a man who is a visiting professor one week can be in the army the next week and can be assigned to checking people's identity papers at the university.

But there are informal collaborations. An African postdoctoral fellow at an Israeli university has been able to conduct a study of a protozoan parasite in West Bank farming villages. But both Palestinian and Israeli scientists are nervous about giving details of the research, for fear that word of the Israeli involvement will get out and the project will be stopped. Only by having a black African graduate student doing the clinical field-work has the project been able to proceed.

There is a small movement within Israeli universities to protest at the closure of West Bank universities. Daniel Amit, a physicist at Hebrew University, has tried unsuccessfully to introduce a resolution to the faculty senate expressing concern about the long-term effects of closing a university based on activities of the

Building medical bridges

ISRAELI hospitals were at first unenthusiastic about training Arab doctors from the West Bank and Gaza Strip as anaesthesiologists when the Military Government asked hospitals to do so three years ago. Although few said so outright, there was a belief that these Arabs — either through carelessness or by intent — might cause injury to Jewish patients undergoing surgery. But the Military Government persisted and, in 1984, 25 Arab doctors went to hospitals scattered throughout Israel. The United Nations and the Israeli Government jointly sponsor the training programme.

At Kaplan Hospital, south of Tel Aviv, chief of anaesthesiology Dr David Soreker trained Drs Jamal Mohammad Mahmoud of Nablus and Yusef Abu Jeries of Beit Jalla. During training, Soreker was pleased not only with what they achieved medically, but also that "they learned to see Jews as human beings, and we to see Arabs in the same way".

After completing their training in Israel — including advanced courses in open-heart and neurosurgical anaesthesiology at Hadassah University Hospital in Jerusalem — Drs Mahmoud and Abu Jeries established departments of anaesthesiology in their local hospitals back home.

After its initial success, the programme is being expanded to include training in neurology, haematology, radiology and oncology. That Palestinian doctors are willing to participate in an Israeli-run programme suggests there is political support for the effort even from Palestinian nationalist organizations.

For Israelis, enlightened self-interest is the motivating factor in participating, says Brigadier (Res.) Arik Beckenstein who helped initiate the programme while serving as Deputy Coordinator in the Administered Areas. "After all", he says, "Arabs and Jews will have to go on living together in this part of the world, whatever political settlement is eventually decided upon".

Nechemia Meyers

Mr Meyers is on the staff of the Weizmann Institute.

students. Amit's colleagues are, for the most part, tolerant of, but not completely comfortable with, his support for Bir Zeit.

Somehow, research on the West Bank manages to continue. Derek Lainé, a professor of physics at Keele University in the United Kingdom, where Yassim did his PhD, arrived for a visit after Bir Zeit was closed. Rather than cancel his trip, Lainé decided to visit anyway, accomplishing what he could in Yassim's living room. "Work goes on", he says of the unfavourable conditions. "You've just got to lump it." □

Relative motions of hotspots in the Pacific, Atlantic and Indian Oceans since late Cretaceous time

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Combinations of global plate reconstructions reveal average velocities for the last 50 to 65 million years of 10 to 20 mm yr⁻¹ between the Hawaiian hotspot and those beneath Iceland, Tristan da Cunha, Réunion, St. Paul's Island, and Kerguelen. Therefore hotspots do not define a fixed reference frame. Uncertainties in these reconstructions are less than the errors incurred by assuming fixed hotspots and less than the differences among various proposed frames of reference of fixed hotspots¹⁻³.

WHEN Morgan^{4,5} elaborated Wilson's⁶ idea that linear volcanic chains and aseismic ridges emanate from localized hotspots, by suggesting that the hotspots are fixed with respect to one another, he offered a hypothesis for defining a fixed frame of reference for plate motions. Although hotspot tracks are now commonly used to constrain histories of plate motion, results of various tests of the fixed hotspot hypothesis differ significantly. By finding agreement between most calculated directions of present-day plate motions and the measured orientations of linear volcanic chains and aseismic ridges, Minster *et al.*^{7,8} found no relative motion among the hotspots for the past 5–10 Myr. Others^{9–11} reported relative motions of hotspots of 10 to 20 mm yr⁻¹ (or more) for longer durations. Morgan^{1,2} questioned these studies, and, like Duncan³, he deduced relative movement of less than 5 mm yr⁻¹ of hotspots in the Atlantic and Indian Oceans. Morgan associated some chains in the Atlantic with different hotspots from those used by Burke *et al.*⁹, and he interpreted the calculated relative positions of hotspots and aseismic ridges differently from Molnar and Francheteau¹⁰.

Because rapid motion of plates over hotspots occurs in the Pacific and not in the Atlantic Ocean, the most definitive test of fixed hotspots must also use the motion of the Pacific plate. In particular, the bend in the Hawaiian–Emperor seamount chain, whose age is 43.1 ± 1.4 Myr (ref. 34), presumably marks a major change in the direction of motion of the Pacific plate. A convincing test of fixed hotspots should thus match the history of plate motions both before and after the formation of the bend, and in particular the position and the age of the bend. Previous attempts to test fixed hotspots could not use the Pacific plate for times older than about 35–40 Myr (ref. 11), because of the obvious existence of a plate boundary somewhere between the North Pacific and East Antarctica (Fig. 1a). We believe that we have found the missing plate boundary in what is now the Antarctic plate¹², so that we can now quantify the displacement at the missing plate boundary and use this to test fixed hotspots since about 68 Myr.

If the motion of one plate over its hotspots is known, and if these hotspots are assumed to be fixed with respect to those under a second plate, reconstructions of the past positions of the two plates can be used to compare the predicted positions of a hotspot under the second plate with its known trace. Because the validity of this test depends on accurately knowing the motion of the first plate with respect to its hotspots, we have carried out two tests. First, using the positions of the African plate with respect to hotspots beneath it^{1–3}, we compared the calculated history of motion of the Pacific plate over the Hawaiian hotspot with the ages and trends of the Hawaiian–Emperor Chain. Second, using those measured ages and trends to define the history of motion of the Pacific plate over the

Hawaiian hotspot, we calculated the histories of motions of the plates beneath the Atlantic and Indian Oceans with respect to their hotspots. We present calculated positions of hotspots for the times of the old edge of anomaly 5 (10.59 Myr), the centres of anomalies 6 (19.90 Myr), 13 (35.58 Myr), 18 (42.01 Myr), 21 (49.54 Myr), and 25 (58.94 Myr), and the reversed polarity interval between anomalies 30 and 31 (68.47 Myr), using the DNAG geomagnetic reversal timescale^{13,14} and recently determined reconstructions for the South Pacific¹², the Indian¹⁵, the South Atlantic¹⁶, and the North Atlantic Oceans (ref. 17; K. D. Klitgord, H. Schouten and P.M., unpublished results). For each reconstruction, uncertainties expressed as partial uncertainty rotations for each pair of adjacent plates¹⁸ have been combined to yield uncertainty ellipses, which we consider to be 95% confidence regions, for the combined rotations¹⁹.

Implicitly we assume that there are no additional plate boundaries at which there was motion of parts of the plates in question. Specifically, we neglect deformation between East and West Antarctica, despite some evidence suggesting a small amount (≤ 200 km) of Cenozoic displacement between them^{12,20}. Thus, we ignore the remote possibility of a small amount of displacement of East and West Antarctica being describable by a large rotation about a pole close to Antarctica.

Motion between Hawaii and Tristan da Cunha

Morgan^{1,2} and Duncan³ concluded that the hotspots in the Atlantic and Indian Oceans move only very slowly with respect to one another. If so, a good test of fixed hotspots could be made by using the history of one plate with respect to its hotspots to calculate the history of motion of the Pacific plate over the Hawaiian hotspot. For Morgan's^{1,2} or Duncan's³ parameters, the calculated motion of the Pacific plate, with respect to a Hawaiian hotspot fixed to the hotspots beneath Africa, changed from southeasterly before 42 Myr to east–southeasterly after that time (Fig. 1). Thus the calculated motions changed in the correct sense and at approximately the same time as the Hawaiian bend, giving us confidence that the modification to the history of the South Pacific¹² is sensible.

None of the three sets of parameters^{1–3} allow the African hotspots and the Hawaiian hotspot to be fixed with respect to one another (Fig. 1b). For Morgan's earlier set¹, the calculated bend lies about 300 ± 100 km from the Hawaiian–Emperor bend, but for both his later set² and Duncan's³, it lies about 800 ± 100 km from the bend. Therefore, both Duncan's³ and Morgan's more recent (presumably preferred) parameters² imply that the average rate of movement between the Hawaiian and Tristan hotspots for the past 42 Myr was 20 ± 2 mm yr⁻¹. These conclusions do not depend upon our revision of the early Tertiary history of the South Pacific. Morgan's older parameters¹ yield

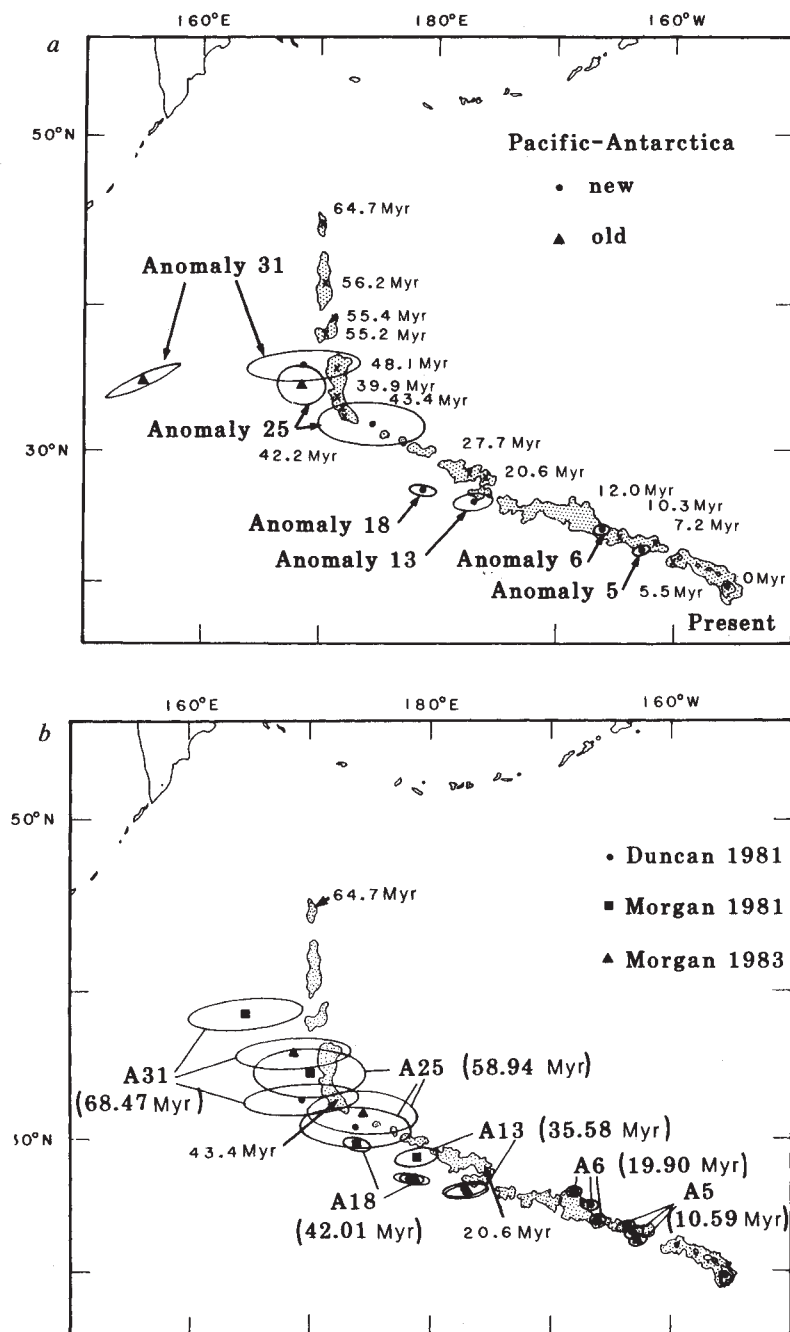


Fig. 1 Maps of Hawaiian-Emperor chain and calculated positions of the Hawaiian hotspot, assuming that it is fixed with respect to the Tristan da Cunha hotspot. *a*, Morgan's² parameters for the motion of Africa over Tristan da Cunha are used, and calculated positions based on the old history of the south-west Pacific and a new, revised version of it¹² are compared. With the old model there is little difference in the directions of relative movement before and after the time of anomaly 18, but with the new one there is. $\times$, indicate selected ages of islands and seamounts³⁴. *b*, Calculated positions using Morgan's^{1,2} and Duncan's³ parameters for the motion of Africa over Tristan da Cunha are compared. Note the significant differences among them for various times before and after the age of the bend. The sequence of rotations is hotspots to Africa¹⁻³, to Antarctica¹⁵ and to the Pacific¹².

a lower average rate: $7 \pm 2 \text{ mm yr}^{-1}$. All three sets of parameters also call for relative motion between these two hotspots since before the formation of the bend.

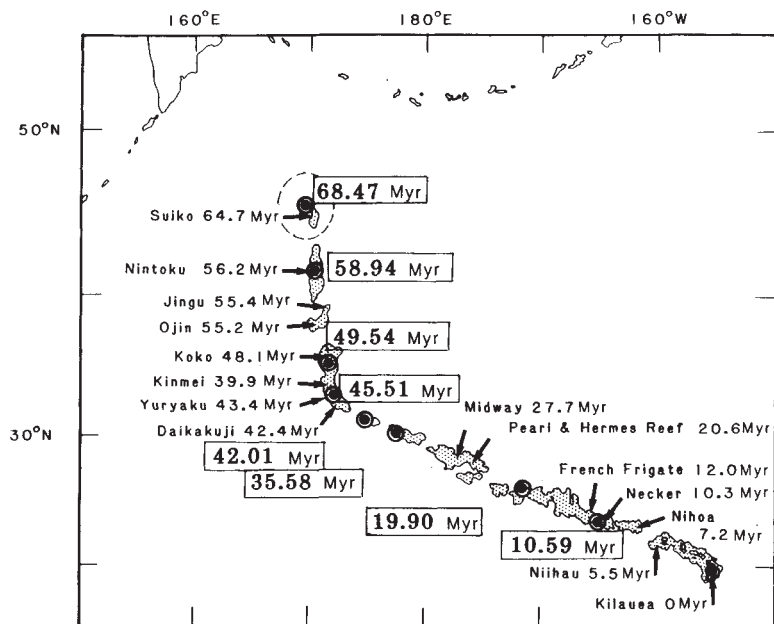
The difference in Morgan's^{1,2} two sets of parameters for fixed hotspots in the Atlantic corresponds to a 500-km difference in the calculated position of the Pacific plate with respect to the Hawaiian hotspot at the time of anomaly 18 (Fig. 1*b*). This difference is both independent of and larger than the uncertainties contributed by the reconstructions in the Pacific-Antarctic-African plate circuit. Clearly the motion of the African plate over its hotspots is not known well enough for it to be used to determine relative velocities among hotspots.

Motion between Hawaii and other hotspots

Because the most thoroughly dated and acknowledged hotspot trace is the Hawaiian-Emperor volcanic chain³⁴, the best test of fixed hotspots can be made by assuming that we know the

motion of the Pacific plate over the Hawaiian hotspot. To describe that motion, we have used poles at 68°N , 75°W (ref. 21) for the Hawaiian Chain (0 to 43 Myr) and at 17°N , 107°W (ref. 24) for the Emperor Chain (43–69 Myr). Rotation angles were derived using Clague and Dalrymple's compiled ages of seamounts and their distances from Kilauea along the Hawaiian-Emperor chain, assuming that the age of the bend is 43.1 Myr (ref. 34) and that its location is 34° from Kilauea²². We allowed for a 95% confidence region of radius 200 km in the positions of the Pacific plate over the hotspot. This spans the width of the chain and at 92 mm yr^{-1} (ref. 34) allows for errors of 2.2 Myr in the ages either of the rocks or of the geomagnetic timescale (Fig. 2). These uncertainties are represented in the combined reconstructions as two partial uncertainty rotations¹⁸: a rotation of 1.94° or 1.87° about the poles, noted above, describing the creation of the Hawaiian or the Emperor chain, respectively, and a rotation of 1.8° about an axis lying 90° from the hotspot in the direction parallel to the chain.

Fig. 2 Map of the Hawaiian-Emperor chain with ages of well dated seamounts and islands³⁴ indicated by arrows and large dots showing the positions of the Hawaiian hotspot at the times of the magnetic anomalies used in calculations of the positions of other hotspots. The dashed circle around the anomaly 31 position shows the 200-km uncertainty assigned to the position of the hotspot and incorporated in subsequent calculations.



Iceland. Volcanism associated with this hotspot presumably is responsible for the southeasterly trending Iceland-Faeroes Ridge and for the British Tertiary volcanic province of igneous rocks with ages of 50 to 60 Myr (ref. 23) (Fig. 3). The Iceland hotspot apparently also underlay Greenland in early Tertiary time; volcanic rock with ages between 55 and 60 Myr (ref. 24) crops out along the coast north of Skaergaard²⁵, where intrusive rock with a Rb/Sr age of 49 ± 2 Myr (ref. 26) is present.

The calculated positions of the centre of Iceland lie progress-

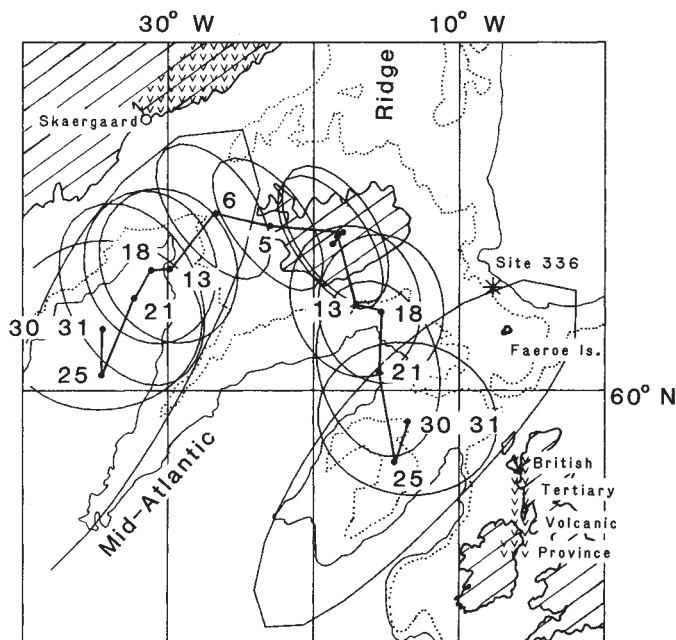


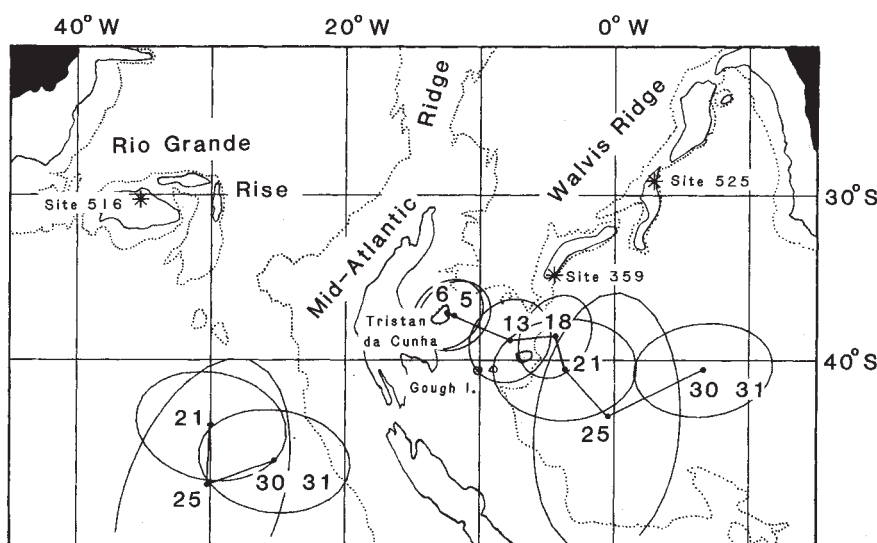
Fig. 3 Map of the North Atlantic showing the calculated positions of the Iceland hotspot, assuming it to be fixed to the Hawaiian hotspot, with respect to Greenland and Europe. The area covered by early Tertiary volcanic rock in Greenland and in the British Isles is shown with Vs. K-Ar ages from basalts at site 336 are 40-43 Myr (ref. 33). The sequence of rotations is hotspots to the Pacific (see text), to Antarctica¹², to Africa¹⁵, to North America¹⁷, to Europe, and to Greenland (K. D. Klitgord, H. Schouten and P. Molnar, unpublished results).

ively south-east of the island at progressively older times and define a trace that diverges from the Iceland-Faeroes Ridge and lies south of it (Fig. 3). Relative movement of the Hawaiian and Iceland hotspots is only barely resolvable. The uncertainty ellipses for the calculated positions of the hotspot overlap the southern edge of the ridge, and if the hotspot had underlain that edge of the ridge, it could have been fixed with respect to the Hawaiian hotspot. In contrast, for about 50 Myr (anomaly 21), the calculated minimum distance of the hotspot from the axis of the ridge is 450 ± 300 km, implying a minimum average rate with respect to the Hawaiian hotspot of at least 9 ± 6 mm yr^{-1} . The calculated positions of the hotspot for about 50 and 59 Myr (anomalies 21 and 25) lie 700 ± 300 km and 600 ± 500 km west of the westernmost volcanic centres in northwestern Scotland and northeastern Ireland. These imply minimum average velocities of 14 ± 6 mm yr^{-1} and 10 ± 8 mm yr^{-1} of the Iceland and Hawaiian hotspots. Finally if, following Morgan^{1,2}, we used the present location of Surtsey, just south of Iceland, instead of the centre of the island, the calculated distances and rates would be about 200 km and 3-4 mm yr^{-1} larger.

The calculated positions of the Iceland hotspot beneath Greenland define a southwesterly trend, instead of the northwesterly trend implied by the outcrops of early Tertiary volcanic rock on the coast of Greenland. The distance from the most southwestern volcanic rock, near Skaergaard, to the calculated positions of the hotspot at about 50 and 59 Myr (anomalies 21 and 25) are 700 ± 200 km and 900 ± 700 km, respectively (Fig. 3). Thus, they imply average velocities between Hawaii and Iceland of at least 14 ± 4 mm yr^{-1} and 15 ± 12 mm yr^{-1} . Again, were the hotspot to underlie Surtsey, the calculated distances and rates would be larger. Moreover, if the distances were measured to the middle of the volcanic area in Greenland, the calculated distances and rates would be another 200 km and 3-4 mm yr^{-1} higher. Thus an average rate of 20 mm yr^{-1} between the Hawaiian and Iceland hotspots is possible.

Tristan da Cunha. The calculated positions of this hotspot define an east-southeasterly trend that diverges markedly from that of the Walvis Ridge (Fig. 4). For 68 Myr (anomalies 30-31), the calculated position of the hotspot lies $1,000 \pm 300$ km from the nearest part of the Walvis Ridge. Therefore it implies a minimum average rate between the Hawaiian and Tristan hotspots of 14 ± 4 mm yr^{-1} . The distance from DSDP site 359, where basalt with an age of 50 to 55 Myr is interpreted to have erupted from a seamount²⁷, to a point midway between the calculated

Fig. 4 Map of the South Atlantic showing the calculated positions of the Tristan da Cunha hotspot, assuming it to be fixed to the Hawaiian hotspot, with respect to South America and Africa. Only calculated positions with respect to South America are shown for the times of anomalies 21, 25 and 30–31, because the Tristan hotspot surely has underlain the African plate since 50 Myr. Note that if the hotspot responsible for the Walvis Ridge and the Rio Grande Rise lay beneath Gough Island, the calculated distance from those features would be 400 km farther than those shown for Tristan da Cunha. The sequence of rotations is hotspots to the Pacific (see text), to Antarctica¹², to Africa¹⁵ and to South America¹⁶, except for anomaly 25, where Antarctica was rotated to India and then to Africa¹⁵.



positions of the Tristan hotspot at about 50 and 59 Myr (anomalies 21 and 25) is 800 ± 500 km corresponding to 16 ± 10 mm yr⁻¹. Tuffs, with a K–Ar age of 40.1 ± 1 Myr, overlie older sediment at this site²⁸. (All K–Ar ages that we quote have not been corrected for new decay constants.) The calculated distance to the Tristan hotspot at 42 Myr (anomaly 18) is 500 ± 300 km. If deposition of these tuffs marked the location of the hotspot, the average velocity would have been 12 ± 8 mm yr⁻¹. The calculated position of the hotspot at 68 Ma lies $1,300 \pm 300$ km from DSDP site 525, where late Maastrichtian sediment (66–75 Myr) overlies basalt²⁹. If the hotspot lay beneath this site at about 68 Myr, the average rate would have been 19 ± 4 mm yr⁻¹. Finally, if the hotspot lay beneath Gough Island instead of Tristan da Cunha, the calculated distances and average rates would be a few hundred km and several mm yr⁻¹ greater.

The calculated positions of the Tristan hotspot with respect to South America for 59 and 68 Myr, lie $1,300 \pm 700$ km and $1,300 \pm 300$ km south of the south-east end of the Rio Grande Rise (Fig. 4). If the Tristan hotspot had underlain the South American plate at those times, minimum average velocities of the Tristan and Hawaiian hotspots would have been at least 22 ± 12 mm yr⁻¹ or 19 ± 4 mm yr⁻¹.

Réunion. Calculated positions of Réunion before 42 Myr (anomaly 18) lie beneath the African plate, but for older times they define a northerly trend a few hundred km east of the Chagos-Laccadive chain (Fig. 5). At 59 Myr, the calculated position is about 500 km east of that chain, but the uncertainty ellipse overlaps part of the chain. The calculated positions for 59 and 68 Myr lie south and east of the Deccan traps, which were erupted at 64 ± 1 Myr (ref. 30) and are thought to result from the Réunion hotspot beneath India¹. The distance from the interpolated position of Réunion at 64 Myr to the southern edge of the Deccan traps is 600 ± 300 km, corresponding to a minimum relative velocity between the Hawaiian and Réunion hotspots of 10 ± 5 mm yr⁻¹. The distance to the centre of the vast area covered by volcanic rock is $1,000 \pm 300$ km, corresponding to an average velocity of 15 ± 5 mm yr⁻¹.

Kerguelen and St Paul's Island. The formation of the Ninety East Ridge has been ascribed to hotspots beneath either Kerguelen or St Paul's Island (or both)³¹, but no volcanic chain or ridge has been found between the southern end of the Ninety East Ridge and St Paul's Island (Fig. 5). Calculated positions of both possible hotspots for 50, 59 and 68 Myr (anomalies 21, 25 and 30–31) define north-northeasterly trends about 1,000–1,500 km east of the Ninety East Ridge. The calculated distances from the Kerguelen and St Paul's hotspots at 59 Myr to DSDP site 214 on the Ninety East Ridge, which yielded K–Ar ages of 52.9 and 53.9 Myr on basalts and biostratigraphic ages on overlying

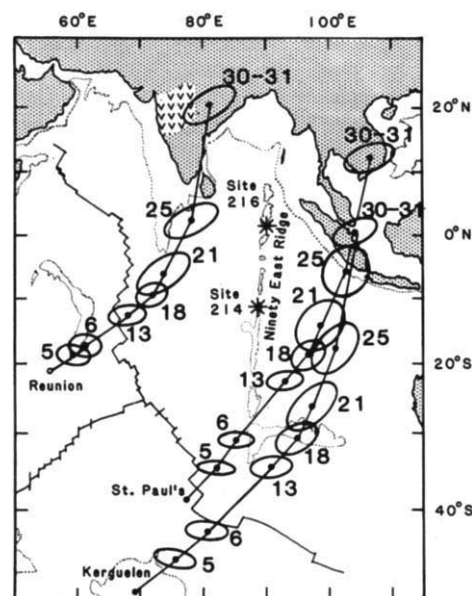


Fig. 5 Map of the Indian Ocean showing the calculated positions of the Réunion, St Paul's, and Kerguelen hotspots with respect to the Indian plate, assuming them to be fixed to the Hawaiian hotspot. The part of India covered by the Deccan traps, with ages of 64 ± 1 Myr (ref. 30), is shown with Vs. The sequence of rotations is hotspots to the Pacific (see text), to Antarctica¹² and to India¹⁵.

sediment of 56–58 Myr (foraminifera) and 57–61 Myr (nannofossils)³² are $1,500 \pm 300$ km and $1,600 \pm 400$ km, respectively. These distances correspond to average rates with respect to Hawaii of 25 ± 5 mm yr⁻¹ and 27 ± 7 mm yr⁻¹. Similarly calculated distances for 68 Myr and DSDP site 216, with K–Ar ages of 62.9, 64.9 and 64.4 Myr and biostratigraphic ages of <67 Ma (foraminifera) and 65–67 Myr (nannofossils)³² are $1,500 \pm 300$ km for Kerguelen and $2,100 \pm 400$ km for St Paul's, corresponding to average rates of 22 ± 4 mm yr⁻¹ and 31 ± 6 mm yr⁻¹. Morgan¹, who did not consider a hotspot under St Paul's Island, referred to Kerguelen as "the least fixed" of the hotspots that he studied in the Atlantic and Indian Oceans. We agree.

Conclusions

Using Morgan's^{1,2} or Duncan's³ motions of Africa over its underlying hotspots, calculated motions of the Pacific plate over

the Hawaiian hotspot show a change in direction at approximately the age of the bend in the Hawaiian-Emperor chain, but the calculated positions of the Hawaiian hotspot for that time are 300 to 800 km from the bend. More importantly, the different parameters¹⁻³ yield very different positions of the bend, implying that the positions of the African plate with respect to the Tristan da Cunha hotspot are not known well enough to be used in a test of fixed hotspots.

Using the history of motion of the Pacific plate over the Hawaiian hotspot defined by the ages of volcanic rocks along the Hawaiian-Emperor chain³⁴, the calculated relative positions of hotspots (presumed to be fixed) beneath Iceland,

Tristan da Cunha, Réunion, Kerguelen and St Paul's Island lie from several hundred to more than a thousand km from the traces left by them. These calculations call for relative velocities between these hotspots and Hawaii of at least 10 mm yr⁻¹ and as much as 20 mm yr⁻¹ in some cases. Thus the hotspot traces do not define a fixed reference frame, and uncertainties in plate reconstructions are much smaller than the errors made in reconstructions based on the assumption of fixed hotspots.

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The crystal structure of *trp* aporepressor at 1.8 Å shows how binding tryptophan enhances DNA affinity

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Comparison of the crystal structure of inactive unliganded trp aporepressor with that of trp repressor shows that binding tryptophan activates the dimer a thousandfold by moving two symmetrically-disposed flexible bihelical motifs. These flexible 'DNA-reading heads' flank a highly inflexible core domain formed by an unusual arrangement of interlocking α-helices from both subunits.

OVER the past several years, the molecular details of transcriptional control have begun to unfold (see refs 1-3 for review). This is particularly true in prokaryotic transcription where improvements in the overproduction of the relevant proteins and large scale oligonucleotide synthesis have provided enough pure material for crystallographic and NMR analysis while genetic manipulation of protein and target DNA sequences have permitted the correlation of mutant phenotypes with *in vitro* biophysical chemistry^{1,4,5}.

The *trp* repressor/operator system is an especially attractive one with which to study the molecular details of high affinity, sequence-specific DNA interaction by genetic regulatory pro-

teins. In addition to an extensive background of genetic studies⁶⁻⁸, one can grow large well-ordered crystals of the *trpR* protein and its important functional variants. These include: 1, aporepressor, the 'inactive' unliganded dimer which, upon binding two molecules of L-tryptophan, is 'activated' to repressor; 2, repressor, the active liganded dimer; 3, the 'inactive' pseudorepressor that is fully liganded with analogues of tryptophan which bind to, and displace, L-tryptophan from its binding pocket⁹; 4, the repressor/operator complex¹⁰ in which the operator is represented by a canonical 18 base-pair twofold symmetrical duplex. The structures of these crystalline molecules when combined with the results of recent site-directed mutational analyses of operator and repressor function¹¹ as well as *in vitro* measurements of repressor/operator affinity should reveal the details of this regulatory system.

In this paper we report the crystal structure determination and refinement of *trp* aporepressor to a nominal resolution of

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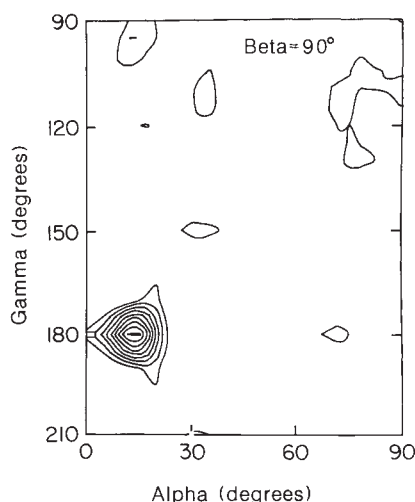


Fig. 1 Rotation Function Map. Fast rotation function of Crowther¹⁷ applied within an integration radius of 25 Å to *trp* aporepressor data from 10 Å to 4 Å. The search structure was the partially refined model to 2.5 Å of the *trp* repressor dimer from which the bound tryptophan and residues 2-11, 107 and 108 were deleted. The contour intervals are equal to the r.m.s. value of the map. The lowest contour is three r.m.s. units.

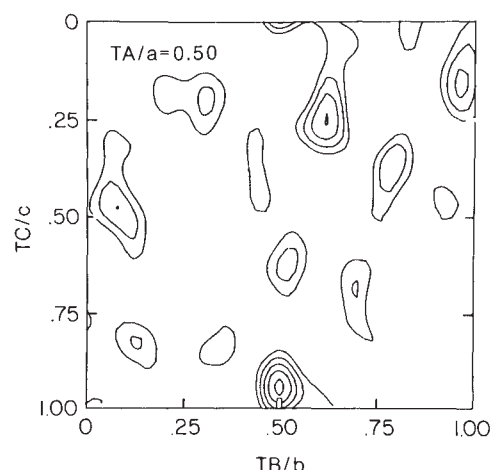


Fig. 2 Translation Function. The TA = 0.5 plane of the translation function map applied to the *trp* aporepressor data from 10 Å to 4 Å resolution, using the model described in Fig. 1, oriented as indicated by the peak therein. The contour interval is 0.8 times the r.m.s. value of the map. The highest peak on this plane is 4.3 r.m.s. units, the highest peak not on this plane is 2.8 r.m.s. units. The lowest contour is one r.m.s. unit.

1.8 Å. The molecular model has been contrasted with the partially refined model of the trigonal crystal form of repressor¹². By contrasting the model of the inactive unliganded aporepressor and the active liganded repressor, we can directly visualize the structural basis for the activating transition and infer from these conformational changes which features are likely to be important in operator binding.

Structure determination and refinement

Aporepressor was purified as described¹⁰ and crystallized using the hanging-drop vapour-diffusion technique¹³. The best crystals were grown at room temperature by equilibrating droplets containing 0.6% protein, 0.8 M ammonium sulphate and 0.4 M sodium, potassium phosphate with a reservoir of 1.85 M ammonium sulphate and 0.5 M sodium, potassium phosphate at pH 7.0. Maximum size (0.1 mm thick) was reached within two months. The crystals were semi-circular, similar in appearance to, but much larger than those reported by Joachimiak *et al.*⁹ and diffracted to 1.8 Å. The space group is P2₁2₁2 with unit cell dimensions of $a = 44.5$ Å, $b = 57.4$ Å, and $c = 34.2$ Å. Assuming standard values for the protein's specific volume (for example, $v = 0.736$ cm³ g⁻¹)¹⁴, the calculated volume of a single subunit is 67.8% of the asymmetric unit. Clearly no more than one subunit can be accommodated in the asymmetric unit, implying that the two subunits of the dimer¹⁵ are related by a lattice dyad, as in the case of the trigonal and orthorhombic crystal forms of the *trp* repressor⁹. A complete data set was collected to 1.8 Å on oscillation photographs and processed by DENZO (Z.O., unpublished).

The crystal structure was solved by molecular replacement¹⁶, applying the forerunner of the program package, MERLOT, provided by Dr P. M. D. Fitzgerald. The partially refined model of the trigonal crystal form of the *trp* repressor was used as the search structure. The *trp* repressor dimer (missing the partially disordered amino-terminal 11 and the carboxyl-terminal 2 residues) was placed in an artificial unit cell (P1, $a = b = c = 110$ Å, $\alpha = \beta = \gamma = 90^\circ$) with the molecular two-fold rotation axis coincident with x . A full rotation-space map was calculated with Crowther's fast rotation function¹⁷ using diffraction data from 10 Å to 4 Å resolution. Figure 1 shows that the orientation of the *trp* repressor model corresponds best to that of the aporepressor when the model structure is rotated by $\alpha = 15^\circ$,

$\beta = 90^\circ$, $\gamma = 180^\circ$. As expected, this places the molecular dyad of the model structure parallel to the lattice dyads.

The model of the repressor was oriented in the orthorhombic cell as indicated by the rotation function and its position was determined by applying the translation function of Lattman¹⁸ to the data between 10 Å and 4 Å. All translation space was searched even though the correspondence of the molecular and lattice dyads requires that the solution fall on the line, TA = 0.5, TB = 0.5; the translation function showed a distinct maximum at TA = 0.5, TB = 0.5 and TC = 0.94 (Fig. 2).

The model of repressor was oriented and translated as indicated by the molecular replacement result, and subjected to constrained/restrained least squares refinement using the program CORELS^{19,20}. Treating the model as a single rigid unit, its orientation and position were optimized, decreasing the R -factor from a starting value of 0.54 to 0.49 for the 354 terms to 5 Å. The six helices (comprising over 80% of the residues) were then constrained as individual rigid groups and the structure refined against the 5 Å data, permitting only the ϕ and ψ rotations in the interhelical segments to vary under firm restraints. This caused the R -factor to decrease to 0.45.

The segmentally-refined model was further refined by the restrained least squares method of Konner and Hendrickson²¹ and periodically adjusted with molecular computer graphics using the model-building program, FRODO²², to fit appropriate difference maps. The general strategy was to proceed from low resolution with tight stereochemical restraints, gradually increas-

Table 1 Refinement summary

R-factor* = $\sum F_o - F_c / \sum F_o $	20.4%
Number of structure factors	6547
Number of non-hydrogen atoms	883
Number of solvent molecules	70
r.m.s. deviation of bond lengths†	0.013 ($\sigma = 0.020$ Å) Å
r.m.s. deviation of interbond angles†	2.48° ($\sigma = 3.07^\circ$)
r.m.s. shift in final cycle	
position	0.010 Å
temperature factor	0.30 Å ²

* For $i > 2\sigma$ and $5.0 \text{ Å} \geq d \geq 1.8 \text{ Å}$.

† r.m.s. deviation = $[\sum_{n=1}^N (x_n - x_0)^2 / N]^{1/2}$ where x_n is the value of the n th parameter and x_0 is the accepted average value from the literature having a standard deviation of σ .

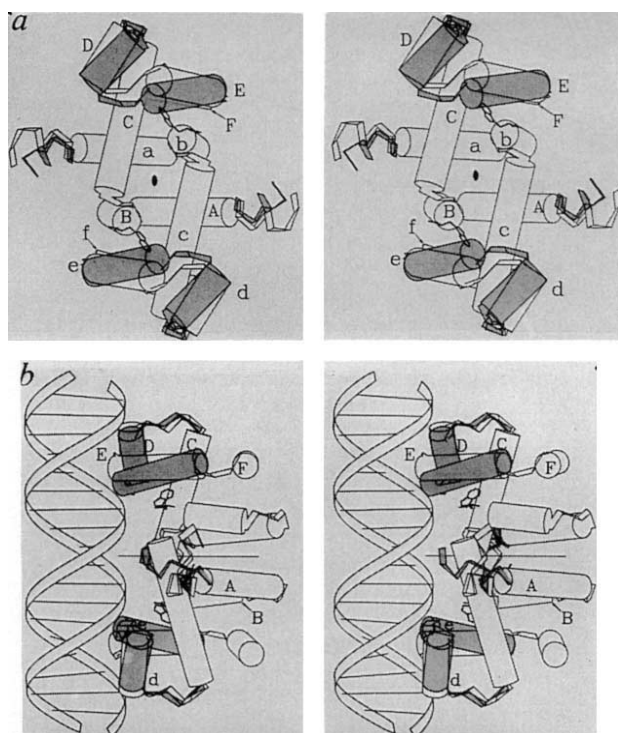


Fig. 3 Comparison of *trp* aporepressor and *trp* repressor. Schematic drawing in which aporepressor was superimposed on repressor so as to minimize the squared differences in position of corresponding α -carbons in the manner described in the legend to Fig. 4. Regions in the aporepressor showing the greatest differences are highlighted by gray shading. Cylinders represent the α -helices designated in Fig. 4. The highlighted regions at the vertical extremes of the model are the flexible reading heads. *a*, View of the DNA-binding surface looking down the molecular dyad. *b*, View perpendicular to that in *a* showing repressor 'docked' to an operator represented as straight canonical B-DNA³⁶.

ing the resolution while relaxing the restraints. The electron density maps used are Fourier syntheses with coefficients $(2|F_o| - |F_c|) \exp(i\phi_c)$ where $|F_o|$ is the structure factor amplitude obtained from the scaled experimental intensity and $|F_c|$ and ϕ_c are the amplitude and phase, respectively, of the corresponding structure factor calculated from the refined model. Segments that appeared poorly defined in these maps, especially those segments of obvious functional significance, were deleted from the model until their structure was clearly indicated by electron density. Water molecules were added at 2.5 Å resolution and the individual isotropic atomic temperature factors of all atoms were refined in the last stages. The current numerical parameters of the refinement are shown in Table 1.

Aporepressor and repressor architecture

Crystals of aporepressor shatter when exposed to L-tryptophan but do not when exposed to its chemically identical but biologically inert enantiomorph, D-tryptophan. This implies: 1, that the molecules in this crystal can bind the corepressor and 2, that the binding and attendant structural changes are strong enough to overcome lattice forces. The crystal structure of the aporepressor described here is, therefore, likely to be a useful representation of the unliganded inactive conformation.

Although the structure of the unliganded *trp* aporepressor is functionally quite distinct, its overall structure is remarkably similar to that of the repressor. In particular, the unique subunit interface composed of interlinked helices is almost perfectly preserved (see below). The unusual character of the activating transition results from a unique, domain structure, in which the dimer undergoes no quaternary changes and the tertiary structural changes are limited to those regions that contact DNA.

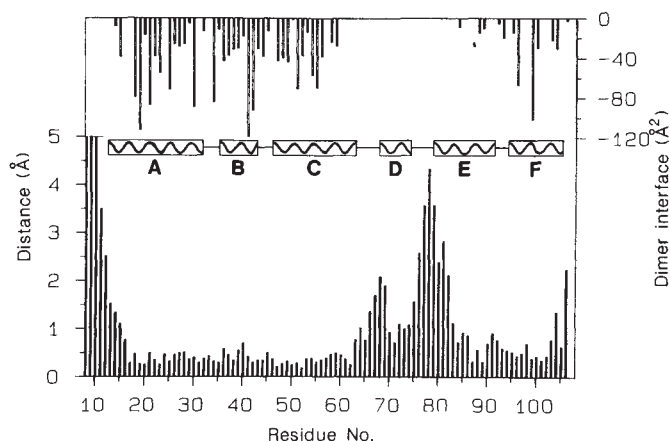


Fig. 4 Conformational differences between *trp* aporepressor and *trp* repressor. A display of the distances between the corresponding α -carbons after the backbone of the two conformers were superimposed with the program PUBFIT (A. Lesk) so as to minimize the square of these distances. The matching process was repeated until all pairs used in the superpositioning were separated by less than one standard deviation (0.6 Å). The occurrence of α -helices is shown as a guide to the sequence³⁷ and to Fig. 3. The upper panel indicates the contact surface for each of the residues forming the dimer interface by showing the increase in solvent-excluded surface area caused by residues in the opposing subunit.

Three domains

To understand the structural transition that converts the 'inactive' unliganded aporepressor to the active liganded repressor, it is best to consider the dimeric aporepressor/repressor structure as three domains. There is a solid 'central core' formed by most of the amino-terminal half of both subunits. This central core is symmetrically flanked by two flexible DNA-reading heads, each formed from the carboxyl-terminal half of one subunit. Figure 3 shows the aporepressor in this three-domain format.

The basis for this structural organization is shown in Fig. 4. When the aporepressor structure is superimposed on that of the repressor so as to minimize the average distance (squared) between the corresponding C_α atoms, the positions of residues 16–65 and 90–104 are indifferent to the presence or absence of bound L-tryptophan. All of the differences in conformation between aporepressor and repressor are restricted to the first 15 amino acids and the contiguous segment from residue 66 to 86. The segments from position 16 to 65 and 90 to 104 that show little conformational change on ligand-induced activation are the same segments that forms most of the interface between subunits (Fig. 4). Schevitz *et al.*¹² have pointed out that this interface is unusual in that most of it is formed by interlocking helices A, B, C and F of both subunits, producing an unusually large contact surface between subunits. In contrast to most other proteins, in which the individual subunits each form a globular structure, both subunits of *trp* aporepressor (and repressor) fold most of the residues of the dimer interface into a common globular domain that resists tertiary and quaternary conformational change. We refer to this firm, unified, globular domain as the central core. The core domain does not appear to interact with DNA, rather it provides a means by which the subunits are rigidly cemented together over an extensive bonding surface. The lack of any quaternary structural change on binding L-tryptophan is consistent with the observation that two corepressor ligands bind to one molecule of the aporepressor without cooperativity^{23–25}.

In contrast to the residues that form the solid central core, amino acids 66–86 show significant tertiary structural changes on binding L-tryptophan. Figure 3 shows that these residues form part of two identical and symmetrical disposed domains

that flank the central core and whose intrinsic conformation and orientation respond to the presence or absence of the corepressor ligand, L-tryptophan. The flanking domains contain helices D and E which comprise the bihelical motif that has been implicated in many DNA binding regulatory proteins as the basis for their specific recognition of, and enhanced affinity for, cognate DNA sequences^{26,27}. Indeed, three of the five largest alpha-carbon shifts in the tryptophan-induced transition occur in the three residues (Ile 79, Ala 80, Thr 81) that modelling studies¹² and genetic analyses^{11,28} indicate to be the most important in operator recognition. We refer to these two symmetrically disposed flanking subunits as the flexible DNA-reading heads. Each is formed by a segment of polypeptide from only one subunit. Their tertiary structure is determined by intrasubunit interactions and is modulated by the presence or absence of bound tryptophan. As shown in Fig. 4, the amino-acid side chains that comprise the reading heads (65–86) do not contribute to the dimer's interface.

The two tryptophan-binding sites lie on each side of the central core, wedged between the solid central core and the flexible reading heads. It appears that the molecule's dimeric architecture is designed to forego quaternary structural adjustments in favour of a solid spacer that separates the tryptophan binding sites and provides a firm platform for independent tryptophan-induced movements of the two flanking domains. The reading heads alone respond independently to the presence of bound tryptophan and provide a conformation that recognizes the appropriate DNA sequence and thereby enhances operator-specific binding. The details of this ligand-induced structural transition are described below.

Mobile amino-terminal arms

The significance of large conformational changes in the amino-terminal 15 residues is not yet clear (Figs 3 and 4); however, they are unlikely to be part of the tryptophan-induced transition. We know that the conformation of the amino-terminal residues in the trigonal crystal form of the repressor is quite different from that in the orthorhombic crystal form, even though the crystallization conditions are virtually identical. It would seem that the structure of the first 15 amino acids is not determined by intramolecular interactions in either the repressor or aporepressor, but is defined largely by interactions with neighbouring molecules in the lattice. As the amino-terminal 15 residues are apparently not part of the protein's globular fold, it is likely that they are required either in an early step of the folding process and/or in DNA binding. The latter function would correspond to the role of the crystallographically disordered amino-terminal eleven residues of *cI* repressor of phage λ ²⁹. Although in the case of the *trp* repressor there is still no genetic or chemical evidence bearing on their functions, the amino-terminal segments, when fully extended, can reach the operator in the docking experiment described by Schevitz *et al.*¹². One might imagine that such a poorly constrained segment of polypeptide chain could be used to stabilize functional contacts with DNA and/or other proteins.

A DNA-binding surface

Except for the amino-terminal 15 residues, the principal shifts that occur upon ligand binding involve sequence positions 67–86. These comprise most of the helix-turn-helix motif and all of the substructure that we propose interacts in a sequence-specific way with the operator^{10,12}. Figure 5 depicts the way in which L-tryptophan induces a structural transition in each of the flanking domains, schematically contrasting a tryptophan-binding site when tryptophan is present (repressor) and when it is absent (aporepressor).

In repressor there is a snug pocket that holds the indole ring of the bound tryptophan. The pocket resembles a sandwich in which the 'top' is formed by the C α of Gly 85 and the proximal three methylene groups of Arg 84, and the bottom is formed by

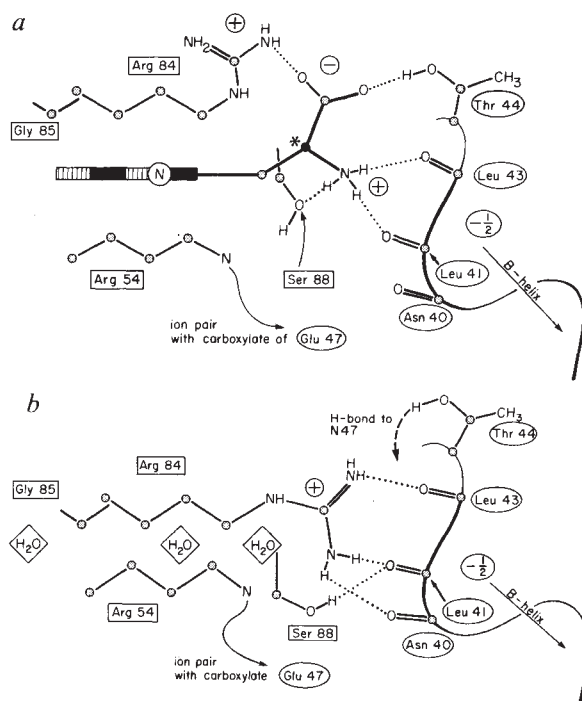


Fig. 5 Tryptophan-binding site. Schematic drawing of tryptophan-binding site in *a*, *trp* repressor and *b*, *trp* aporepressor showing the changes in the immediate neighborhood of the ligand-binding site that underlie the aporepressor/repressor transition. Rectangular and oval labels distinguish the two subunits.

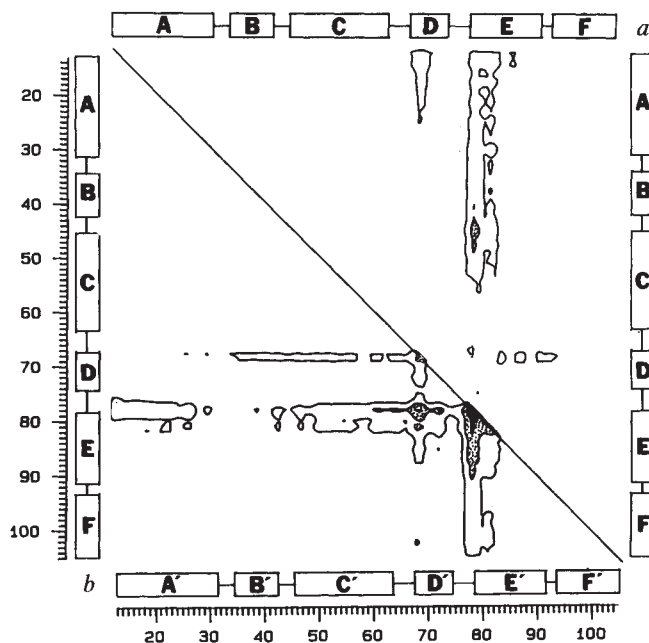


Fig. 6 Difference distance matrix for repressor minus aporepressor. Positive shifts are contoured with solid lines, negative shifts with dotted lines. The first contour is ± 2 Å, above 4 Å (stippled) and 6 Å (black). Boxes labelled A–F represent the helices of one subunit, and those labelled A'–F' represent the helices of the dyad-related subunit. *a*, Interatomic distance shifts within one subunit; each matrix element *i, j*, represents the distance between α -carbon atoms *i* and *j* within one subunit of repressor minus the distance between atoms *i* and *j* within one subunit of aporepressor. *b*, Interatomic distance shifts between subunits. Each matrix element *i, j* represents the distance between α -carbon atom *i* of one repressor subunit and α -carbon atom *j* of the opposing subunit minus the corresponding distance in aporepressor.

the proximal three methylene groups of Arg 54. Gly 85 and Arg 84 are part of the E-helix, the second helix in the bihelical motif, whose amino terminal residues have been implicated in operator recognition^{1,12,28}. The E-helix is a critical element in the flexible reading head, and because it forms the top of the pocket, its orientation and position depend on the presence or absence of the bound tryptophan's indole ring. The 'bottom' of the sandwich is formed by the side chain of Arg 54 that is extended and immobilized in a hydrogen-bonded ion pair to the carboxylate of Glu 47 in the opposing subunit. The Arg-54-Glu 47 ion-pair at the bottom of the indole pocket is typical of the interfacial structure between subunits that forms the solid central core, as it retains exactly the same conformation whether or not tryptophan is bound. When the corepressor ligand is absent, Gly 85 and especially Arg 84 of the E-helix collapse into the resulting vacancy, causing the amino-terminal end of the E-helix to tilt (and to a small extent, bend) towards the molecular dyad, dragging the D-helix and the intervening turn with it (Fig. 3). In addition to the overall shift of the bihelical motif, there is a smaller conformational change within the reading head in which helices D and E change their orientation with respect to each other (Figs 6, 7). The net result of removing the bound L-tryptophan is to cause a substantial change in the repressor's DNA-binding surface (Fig. 3). It is evident from Fig. 3b that the snug fit of the repressor's reading head (Ile 79, Ala 80, Thr 81) with the major groove of the operator cannot be made with aporepressor, nor can possible supplementary specific polar interactions be made by residues in the D-helix¹². In contrast to helix D and E (and their connecting loops), the state of ligation does not affect the conformation of the central core; in fact, helix F of the central core acts as a stable pivot point that anchors the carboxyl terminus of the E-helix during the large tilting movements of the reading heads.

Figure 5 also shows that the guanidino group of Arg 84 in the aporepressor occupies the position taken by the bound tryptophan's α -amino group in the repressor. Schevitz *et al.*¹² noted in their report of the crystal structure of *trp* repressor that the amino group of the bound L-tryptophan formed hydrogen-bonds to carboxyl groups 41 and 43 at the carboxyl-terminus of the B-helix, and suggested that the apparent role for the charged α -amino group in the corepressor ligand was to mitigate the negative pole of the B-helix's intrinsic dipole which could otherwise repel the nearby sugar-phosphate backbone of bound DNA. They further suggested that in the absence of the corepressor the Arg 84 side chain would replace the α -amino group of the ligand. Figure 5 confirms this suggestion; in the absence of the activating ligand, the guanidino group of Arg 84 reaches across the vacancy and forms hydrogen bonds with two carbonyl groups of the carboxyl terminus of the B-helix. Like the corepressor's α -amino group, its positive charge mitigates the partial negative charge of the B-helix's dipole³⁰. This preservation of an electrostatically similar surface irrespective of the state of ligation may reflect a need to maintain an attractive electrostatic interaction between aporepressor and DNA, such as that proposed for models of one-dimensional diffusion³¹.

In summary, it would appear that the appropriate shape and orientation of the repressor's reading head is a critical feature required for operator-specific binding, and it is this conformation and only this conformation that is modulated in the aporepressor-repressor transition.

A hydrophobic brace

It has already been noted by others^{32,33} that the intrinsic conformation of this motif, as well as its orientation in relation to the rest of the molecule is fixed by a cluster of hydrophobic residues referred to here as the 'hydrophobic brace'. Table 2 shows the members of this cluster in the protein product of the *trpR* gene, *cro* repressor, *AcI* repressor and CAP, and emphasizes the spatial correspondence of the contributing residues of the helix-turn-helix. Figure 7 illustrates that in *trp* aporepressor and repressor

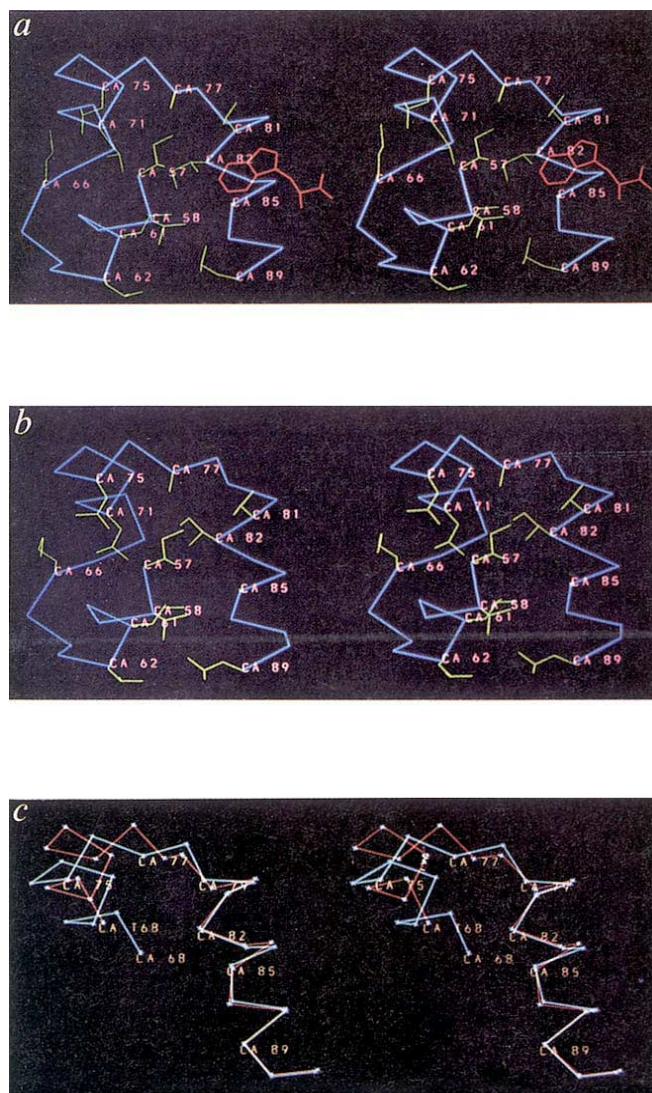


Fig. 7 Hydrophobic brace of the bihelical motif. Stereoviews of the residues that stabilize the helix-turn-helix in *a*, *trp* repressor; *b*, *trp* aporepressor. The reader is viewing the reading head from a point just central to the tryptophan binding site; about the level of Arg 54. Bound tryptophan is highlighted in red. The side chain of Ile 82 is disordered in *trp* repressor and modelled into an arbitrary but stereochemically acceptable position. *c*, A comparison of the relative orientation of the D and E helices in the repressor (red) and aporepressor (blue). Numbers (except T68) correspond to α -carbons of aporepressor. E-helices are superimposed to fit alpha carbons 79–91. The helix-turn-helix is viewed as in *a* and *b*.

the bihelical motif's conformation is maintained by two different arrangements of side chains in the hydrophobic brace. As described above, the conformation of the flexible reading heads depends on the presence or absence of L-tryptophan. Because *trp* repressor modulates its DNA affinity in response to bound L-tryptophan, it is reasonable to expect that in the *trp* repressor the corepressor ligand would contribute to the hydrophobic brace that stabilizes the 'active' conformation of the helix-turn-helix. It is equally important that in the absence of L-tryptophan the remaining elements of the brace must favour an alternative inactive conformation with different stabilizing interactions. The stereochemical basis of this transition can now be described.

The bound tryptophan's indole ring occupies a site near Gly 85 in *trp* repressor. In almost all analogous DNA-binding proteins Gly 85 is replaced by an amino acid with a large hydrophobic side chain that contributes to the hydrophobic brace; in almost half of the cases the amino acid is tryptophan. In the *trp* repressor

Table 2 Components of the hydrophobic brace

Proteins	Helix 1					Turn			Helix 2			r.m.s. Δ
	57	58	61	62	66	71	75	77	82	85	89	
<i>trpR</i> protein	Ile	Val	Leu	Leu	Leu	Leu	Leu	Ala	Ile	Gly	Leu	
<i>cro</i> repressor†	42	40	7	6	14	19	23	25	30	33	37	0.68 Å
	Leu	Ile	Leu	Thr	Phe	Thr	Leu	Val	Ile	Ala	Gly	
CAP†	143	144	147	148	167	172	176	178	183	186	190	0.98 Å
	Ile	Ala	Leu	Leu	Ile	Ile	Val	Cys	Val	Ile	Ile	
λ C1 repressor†		65	51	18	31	36	40	42	47	50	15	0.96 Å
		Leu	Phe	Leu	Leu	Val	Met	Met	Val	Leu	Ala	

Residues forming the hydrophobic brace that occur in the bihelical motif are shown in bold. The remaining residues occur outside the bihelical motif. Entries in a vertical row indicate rough spatial equivalence of the residues having side chains that occupy about the same position when the corresponding α -carbons of the bihelical motif are superimposed by PUBFIT. The r.m.s. Δ is the root mean square discrepancy in position of the corresponding alpha carbons of the bihelical motif when fit to *trp* repressor. The values are upper limits since some of the discrepancies probably reflect errors due to incomplete refinement.

* In *trp* repressor the α -carbon of Gly 85 is in contact with the indole ring of the bound repressor.

† α -carbon coordinates for the *cro* repressor, CAP and λ C1 repressor were obtained from Ohlendorf *et al.*³⁸, the Brookhaven Data Bank/I. Weber, T. Steitz, C. Pabo and S. Jordan (private communication), respectively.

the activating ligand can be accommodated only if space is provided by having glycine at position 85. Thus the bound tryptophan serves as a removable lynch pin in the hydrophobic brace of the active *trp* repressor that, when pulled out, causes the active conformation of the reading head to become unstable and collapse into a stable but inactive aporepressor conformation. In the unliganded aporepressor, the space vacated by the indole ring of the corepressor is filled by: 1, the proximal three ethylene groups of the repositioned side chain of Arg 84; 2, the alpha-carbon of Gly 85; and 3, three entrapped water molecules.

The conformational changes in the reading heads caused by the binding (or loss) of tryptophan is of two types. The dominant shift is caused by an en bloc collapse of the heads toward the dyad axis in the absence of the corepressor ligand. Although this is depicted in Fig. 3, it is shown more quantitatively in a 'difference distance matrix'^{34,35} that compares the interatomic (α -carbon only) distances of aporepressor with those of the repressor (Fig. 6). One can immediately see that the largest changes in interatomic distances occur between corresponding atoms in opposing subunits, especially between the reading heads. By far the largest change caused by binding tryptophan is an 8 Å lengthening of the distance between dimer-related α -carbons of Ile 79 at the amino-terminal residue of the E-helix. Spreading apart the reading heads enables them to fit readily into successive major grooves of B-DNA. The ligand-induced differences in the distance between the reading heads is much larger than those internal readjustments that occur within the reading heads (Fig. 6b). The nature of the reshaping of the reading head *per se* is shown in Fig. 7a,b,c. Even though the internal shifts are small in magnitude, they can be of great consequence in providing just the right side-chain interactions to stabilize an alternative conformation.

The sequence feature of the hydrophobic brace that permits this rearrangement of the bihelical motif is the presence of Ala 77 and Gly 78 at the third and fourth positions of the 'turn'. In almost all analogous proteins, one or both of the amino acids at corresponding positions has a bulky hydrophobic side chain. In the active conformation of repressor the small side chain of Ala 77 packs poorly against its hydrophobic neighbours, providing less stability to the brace. This local looseness in side-chain packing is evidenced by the surprising finding that the adjacent buried side chain of Ile 82, a seemingly important contributor to the hydrophobic brace in analogous proteins, is disordered in repressor. The unusually small side chain of Ala 77 apparently allows the reading head to shift out of its active conformation upon the loss of bound tryptophan. This role is supported by the fact that a mutant in which the small side chain of Ala 77 is replaced by the bulkier and more commonly found side chain

of Val 77 (ref. 28), the *trpR* protein does not require tryptophan for operator specific binding. Moreover, in the absence of tryptophan the AV77 protein crystallizes isomorphously with the liganded repressor. Thus, the presence of a bulkier group at position 77 maintains the reading head in an active conformation even in the absence of bound tryptophan. In further support of the idea that the transition requires a small side chain at position 77, we note that the bulkier side chain of Val cannot be accommodated at position 77 in the aporepressor; that is, alanine's smaller side chain is needed for the reading head to achieve the 'inactive' conformation seen in the aporepressor.

In summary, the structural changes in the flexible reading heads involve two alternative arrangements of buried residues in the hydrophobic brace. In the active conformation of the repressor, the indole ring of the bound tryptophan is an integral part of the brace that stabilizes an otherwise unstable conformation; the inactive conformation of the aporepressor is an alternative arrangement of buried hydrophobic side chains which forms a stable compact brace when no tryptophan is present. This conformation of the reading heads in the aporepressor produces a surface that does not complement the recognition surface of the operator. The flexibility needed to achieve the transition is provided primarily by Ala 77 and possibly Gly 78, the third and fourth residues of the 'turn' in the bihelical motif.

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LETTERS TO NATURE

Explosion of a blue supergiant: a model for supernova SN1987A

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We present a model for the outburst of supernova SN1987A. This supernova was discovered in the Large Magellanic Cloud on 24 February, 1987. Astrometry^{1,2} reveals the supernova to lie within 0.1 arcs of the B3 I supergiant Sanduleak (Sk) -69 202. The optical features indicate that the event was a type II supernova. Such supernovae are the expected consequence of the evolution of massive stars $M \geq 10 M_{\odot}$ and may be expected to leave behind a neutron star or black-hole remnant. Neutrinos released as a consequence of the anticipated core collapse to neutron star densities may indeed have been observed (ref. 3; Aglietta *et al.* preprint, 1987; Bionta *et al.* preprint, 1987). SN1987A certainly cannot be considered a prototype of a type II supernova, however, as it has proved to be unusual in several respects, including: (1) the progenitor of SN1987A appears to have been a blue supergiant rather than a red supergiant star; (2) SN1987A was found to be some 3 or 5 magnitudes dimmer at visual maximum than is known to be typical of supernovae of type II; and (3) SN1987A exhibited an extremely rapid spectral development, seemingly consistent with the very high expansion velocities inferred from studies of the hydrogen absorption features in the early spectra. Here we present a model for the outburst of SN1987A that permits a straightforward interpretation of the observed behaviour.

A particularly important early clue to the nature of this supernova event was provided by the probable identification of the progenitor of SN1987A with the B3 I supergiant star Sk -69 202. Such behaviour was not anticipated by supernova theorists, because standard models for the expected massive star ($M \geq 10 M_{\odot}$) progenitors of type II supernovae generally predict that they should evolve to M supergiants before the ignition of core carbon burning and thus subsequently explode as M supergiants. The fact that this did not occur holds important implications for the evolution of this supernova through outburst.

It is interesting to note that ultraviolet observations in the first few days of the outburst revealed the gradual re-emergence of an ultraviolet spectrum compatible with that of a blue supergiant, which led to the suggestion that the supergiant star Sk -69 202 might still be there and therefore that it was not

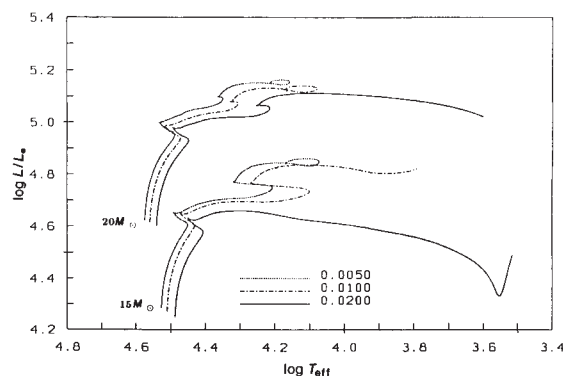


Fig. 1 Evolutionary tracks for $15 M_{\odot}$ and $20 M_{\odot}$ stellar models from the main sequence to the onset of central carbon burning for different metallicities Z . A helium concentration of $Y=0.26$ was assumed. The model with $M=15 M_{\odot}$ and $Z=0.02$ has been followed only up to the ignition of central helium burning.

the site of the outburst⁴. However, ultraviolet spectra taken after March 1987 are fully consistent with the conclusion that the brightest component has indeed disappeared⁵. While further observational work will hopefully be able to establish with absolute certainty the identity of the progenitor of SN1987A, we think it reasonable to assume at this stage that it was indeed a blue star.

A possible explanation for the occurrence of a blue supergiant progenitor is provided by the fact that this supernova is associated with a metal-deficient stellar population. Stellar evolution calculations by Brunish and Truran⁶ predict that stars of ~ 15 – $20 M_{\odot}$ of low metallicity can reach the onset of carbon burning as blue supergiants. Specifically, a model star of mass $15 M_{\odot}$ and composition $Z=0.001$ was found to ignite carbon at a luminosity $\sim 8 \times 10^4 L_{\odot}$ and an effective temperature $\sim 13,000$ – $16,000$ K, compatible with the characteristics of the putative progenitor of SN1987A, Sk -69 202. Additionally, this result was found to be quite insensitive to assumptions concerning mass loss.

Our stellar evolution calculations, performed using the program of Kippenhahn, Weigert and Hofmeister⁷ as modified by Weiss⁸, confirm this general behaviour: significantly, we find that a star of $15 M_{\odot}$ ignites carbon burning as a B supergiant even for a metallicity of $Z=0.005$ ($\approx Z_{\odot}/4$), consistent with that of the Large Magellanic Cloud (LMC). Depending on the treatment of convection, it may also be found that stars in the mass range ~ 15 – $25 M_{\odot}$ can reach the supernova stage as blue supergiants even for higher metallicities. Figure 1 shows the evolutionary tracks of model stars of 15 and $20 M_{\odot}$ for metal-

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licities $Z_{\odot}$, $Z_{\odot}/2$ and $Z_{\odot}/4$. We note that our $15M_{\odot}$ model with $Z=0.01$ remains somewhat bluer than the corresponding Brunish and Truran model. Furthermore, our evolutionary tracks often show reddish loops after the end of core hydrogen burning. These differences from Brunish and Truran's results can be attributed to our use of different opacities⁹ and their influence on the envelope structure. In general, the evolutionary tracks for stars in this mass range are quite sensitive to such details as opacity and the treatment of convection¹⁰.

Stars in this mass range, $\approx 15\text{--}20M_{\odot}$, for metallicities consistent with the LMC, are characterized, at the onset of core carbon burning, by luminosities and effective temperatures in the range inferred for Sk-69 202. As the thermal timescale is less than or comparable to the nuclear burning timescale, we anticipate that no significant further evolution to the red will occur. We therefore find the occurrence of a blue supergiant progenitor of a type II supernova to be a quite understandable phenomenon. Given the inferred spread in metallicities for the LMC (C. Chiosi, personal communication), this can be understood without causing any significant problems with respect to the observed distribution of blue and red supergiants in the LMC.

Proceeding on the assumption of a blue supergiant progenitor for SN1987A, we wish to demonstrate that the observed photometric and spectroscopic evolution over the first few weeks of outburst can be understood in the context of this model. To obtain more detailed information to be used to check our supernova model with observations, we have calculated synthetic spectra for representative supernova envelope models. The observed spectra are mainly dominated by hydrogen features and the envelope is at low densities and these facts lead us to allow for the possibility that the first eight levels of hydrogen may deviate from local thermodynamic equilibrium (LTE). Both in the statistical equations and in our treatment of radiative transfer, all bound-free and 14 bound-bound transitions are included. In addition, both free-free and Thomson-scattering opacities are taken into account. In the radiative transfer equation, the opacities and the source functions of the higher members of the Balmer series ($n \geq 9$) are approximated by the Balmer continuum. This is a reasonable approximation as the occupation numbers for levels $n \geq 6$ are near LTE. We use this approximation because the U colour is strongly influenced by the hydrogen-line blanketing. Due to the fact that the timescale for radiative cooling is short with respect to the hydrodynamic timescale, we can reasonably make the assumption of radiative equilibrium to determine the temperature structure of the models. Finally, we define the photospheric radius $R_{5,000}$ as the radius at which the optical depth for true absorption in the continuum at $5,000 \text{ \AA}$ is equal to one.

Guided by the stellar evolution calculations discussed above, we have assumed a progenitor model of $15M_{\odot}$. We call attention to the fact that stellar evolution calculations predict that B supergiants stars in the mass range between 10 and $25M_{\odot}$ are nearly homologous. Therefore, the resulting density structure should be approximately independent of the exact mass of the progenitor.

To model the dynamics of an expanding supernova envelope without performing a complete set of radiation hydrodynamic computations we proceed as follows. According to detailed numerical models¹¹ and also analytical solutions for strong shock waves¹² the density structure in a spherically expanding envelope is approximately given by a homologous expansion

$$r = \lambda(t)R \text{ and } \rho(r) = \lambda^{-3}\rho(R)$$

where r is the radial coordinate of a given mass element at time t , R its distance from the centre of the explosion at an initial time t_0 , and $\rho(r)$ and $\rho(R)$ are the corresponding mass densities. Finally, $\lambda = \lambda(t)$ is the expansion factor. In a strong shock wave the density in the shock is increased by a constant factor relative to its pre-shock value and because we are interested in timescales

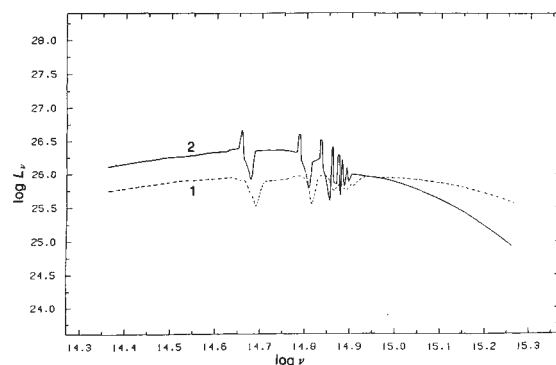


Fig. 2 Calculated luminosities as a function of the frequency ν for models corresponding to about 0.9 and 2 days after the initial event. Curve 1: $T_{\text{eff}} = 14,000 \text{ K}$, $R_{5,000} = 1 \times 10^{14} \text{ cm}$, $v(R_{5,000}) = 21,500 \text{ km s}^{-1}$; curve 2: $T_{\text{eff}} = 9,100 \text{ K}$, $R_{5,000} = 2.4 \times 10^{14} \text{ cm}$, $v(R_{5,000}) = 17,000 \text{ km s}^{-1}$.

much longer than the propagation time of the shock to the photosphere of the progenitor star, we will assume that the density profile at time t_0 can be approximated by that of the progenitor. We will further assume that the kinetic energy of each mass element is conserved during the expansion which is justified by the fact that the observed velocities are significantly higher than the escape velocity. Consequently, $\lambda (=d/dt(\lambda))$ has to be constant in time and can be determined from the observed velocities. We find that $\lambda \approx 9 \times 10^{-4} \text{ s}^{-1}$ fits the observations. Once the expansion law has been fixed the only free parameter in our model is the luminosity in the V-band, M_V , at various times. By taking observed values for M_V we can determine $R_{5,000}$ as well as the total bolometric luminosity and the spectrum for our model atmosphere. The effective temperature T_{eff} then follows from the relation $L_{\text{bol}} = \text{constant} \times R_{5,000}^2 T_{\text{eff}}^4$.

From our model we find that the effective temperature T_{eff} decreases and $R_{5,000}$ increases very rapidly from February 24.3 ($T_{\text{eff}} \approx 14,000 \text{ K}$; $R_{5,000} \approx 1 \times 10^{14} \text{ cm}$) to February 25.5 ($T_{\text{eff}} \approx 9,100 \text{ K}$; $R_{5,000} \approx 2.4 \times 10^{14} \text{ cm}$). It then changes rather more slowly over the period from March 1 ($T_{\text{eff}} \approx 6,800 \text{ K}$; $R_{5,000} \approx 5.8 \times 10^{14} \text{ cm}$) to March 7 ($T_{\text{eff}} \approx 4,800 \text{ K}$; $R_{5,000} \approx 9 \times 10^{14} \text{ cm}$) and on through March 14 ($T_{\text{eff}} \approx 4,200 \text{ K}$; $R_{5,000} \approx 1.2 \times 10^{15} \text{ cm}$). The relatively slow increase of the photospheric radius is due to the fact that the matter which is at greater distances than $1\text{--}1.6 \times 10^{15} \text{ cm}$ becomes neutral. For the purposes of illustration, the calculated spectra for the very early stages, 0.9 and 2 days after the core collapse, are presented in Fig. 2. In the first spectrum, virtually no emission component can be seen. The absorption is mainly due to non-LTE effects and is only slightly dependent upon the temperature structure. The observed rapid increase of the emission component is a consequence of the steep gradient in the envelope. The ultraviolet flux is dominated by scattering. Our model explains the observed rapid development and decrease of the ultraviolet flux during the early phases of the evolution as being attributable to the extremely rapid temperature decrease. After the first few days, the ultraviolet luminosity remains nearly constant because the temperature shows only slight changes in time and the optical depth in Thomson scattering remains nearly constant ($\tau_{\text{sc}} \approx 20$ on March 1; $\tau_{\text{sc}} \approx 15$ on March 7). The strong increase in the infrared flux during the first week is attributed to the rapid increase of the photospheric radius. In addition, the observed behaviour of the colour indices $U-B$ and $B-V$ can be reproduced by this model (see Fig. 3). The strong decrease in $U-B$ is mainly due to the increasing line absorption of the higher members of the Balmer series. A more detailed discussion and a quantitative comparison with the observations is in preparation.

To estimate the expected future behaviour of the light curve, it is of interest to determine the amount of envelope matter $\delta M_{\text{envelope}}$ which has passed through the photosphere, recognizing that the ionized region contains only a small fraction of a

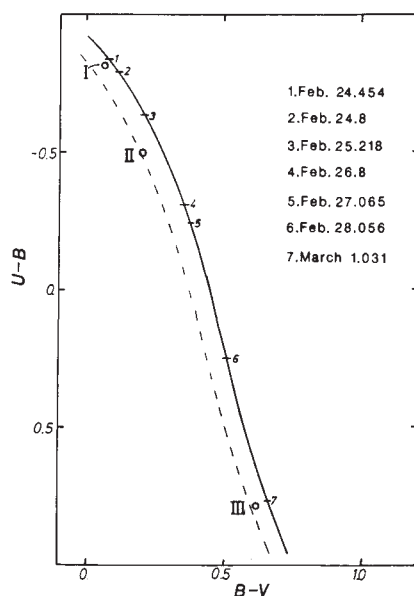


Fig. 3 Comparison of computed colour indices ($U-B$) and ($B-V$) with observations. The solid line indicates the observed colours without reddening corrections; for the dashed curve, we assume a reddening correction $E(B-V) = 0.08$ mag. The theoretical values, shown as open circles, are seen to lie in this defined range. The models correspond to the first three models given in the text.

solar mass at any given time. The models indicate a $\delta M_{\text{envelope}}$ of 0.22, 1.06 and $1.65 M_{\odot}$, respectively, one, two and three weeks after the initial supernova event. Subsequently, the density structure in the envelope will change more slowly because of the structure of the adopted blue progenitor. If we assume a total hydrogen envelope mass of approximately $10 M_{\odot}$ to be involved, then the spectrum may be expected to remain nearly constant over a period of at least two to four months after the initial outburst, with the exception of a decreasing photospheric velocity.

We have thus established that many of the observed features of SN1987A through outburst can be explained on the basis of our model. In addition, such models involving an initial radiative envelope structure can readily explain both the absorption components in the early stages of evolution as due to non-LTE effects and the rapid occurrence of P-Cygni profiles after only 1–2 days. In contrast, we find that the rapid development of the spectrum cannot be readily understood if we assume that the supernova progenitor was characterized by a convective envelope structure compatible with that of an M supergiant. In particular, the strong P-Cygni profiles would first be observed at significantly larger radii ($R_{5,000} \geq 10^{15}$ cm) at a correspondingly later time after the initial explosion.

We also note that models involving an initial radiative envelope structure are consistent with the observation that SN1987A was considerably dimmer at visual maximum (by some 3 or 5 magnitudes) than typical type II supernovae. The significantly higher densities at the base of the envelope imply a correspondingly longer diffusion timescale, and thus allow the possibility that a significantly larger fraction of the outburst energy will be realized in kinetic energy of mass motions at the expense of the photon luminosity.

SN1987A clearly represents an extraordinary event for study by supernova theorists. It represents the first opportunity to identify clearly a type II supernova progenitor star with a massive star. Before this event, it was a matter of considerable debate whether massive stars would give rise to type II supernovae and neutron star or black-hole remnants.

The fact that the progenitor star was a B3 supergiant restricts the mass to $M \leq 20 M_{\odot}$, compatible with our model assumptions. Indeed, we have demonstrated that an entirely consistent expla-

nation of the observed behaviours of SN1987A in outburst is provided by the assumption that the progenitor was a blue supergiant of mass 15 to $20 M_{\odot}$. It appears that a significant clue to this occurrence may be the fact that the metal content of the LMC is low relative to the Sun, for which it follows from stellar evolution calculations that stars in this mass range can reach the endpoints of their active burning lifetimes as blue supergiants. Indeed, models of mass $15 M_{\odot}$ and metallicities $Z \leq 0.25 Z_{\odot}$ exhibit luminosities and effective temperatures at the onset of core carbon burning which are consistent with Sk-69 202.

From our model we can understand, for example, that (1) the progenitor of SN1987A was a blue supergiant; (2) the observed velocities are somewhat higher than the typical values for type II supernovae; (3) SN1987A was significantly dimmer at visual maximum than typical type II supernovae observed in normal spiral galaxies; (4) the bolometric luminosity has remained approximately constant over a period of weeks; (5) SN1987A exhibited an extremely rapid spectral evolution at both optical and ultraviolet wavelengths compared with other observed type II supernovae. The significant feature here would appear to be the steeper density gradient characteristic of blue supergiant stars (as opposed to red supergiants) with radiative envelope structures. Interestingly, L. B. Lucy (personal communication) finds that both ultraviolet and optical line spectra of SN1987A shortly after outburst are quite accurately reproduced with the assumption of an expanding spherical shell with a power-law dependence of gas density on radius compatible with that of a blue supergiant progenitor. This result is supportive of our model for SN1987A.

The mass range 15– $25 M_{\odot}$ (for which we find that a blue supergiant progenitor can be expected in a system of metallicity compatible with the LMC) is consistent with either neutron star or black-hole formation¹⁵ and thus with the occurrence of such neutrino events as have been reported by the Mont Blanc (Aglietta *et al.* preprint, 1987), Kamiokande³, and IMB (Bionata *et al.* preprint, 1987) neutrino experiments. Furthermore, we expect heavy elements to be formed in significant amounts^{14,15}. It is also interesting to consider the question of the 'observable' rate of type II supernovae in low metallicity populations. Because the greater fraction of type II supernovae occurring in low metallicity populations in irregular galaxies like the LMC may behave like SN1987A, and be considerably less luminous at visual maximum than the type II supernovae which are observed in spiral galaxies, this model may explain why we have not previously observed a type II supernova event in an irregular galaxy. It may also imply that the average rates of type II supernova activity over the lifetimes of normal galaxies have been higher than previously estimated.

Our model also makes several predictions concerning the subsequent evolution of the light curve and spectrum of SN1987A. We expect that the general behaviour of the spectrum should not change significantly over the next few months, except that the observed velocities may be seen to decrease. The supernova should continue to evolve at approximately constant bolometric luminosity over this same period, providing that the deposition of shock energy per unit mass was constant over the envelope. Finally, changes in the spectrum should ultimately reflect the heavy-element-rich composition of ejected core matter on a timescale of months. The presence of a young neutron star remnant would of course ultimately influence the evolution of the expanding supernova shell.

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Light curve asymmetry of a relativistic accretion disk

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The emergent radiation from accretion disks has been observed in several objects. For instance, the double-peak spectra of Balmer lines emitting at the outer disk have been found in cataclysmic variables^{1,2}. The continuum radiation from quasars was fitted with the spectra of standard accretion disk models³. Furthermore, the X-ray spectra from low-mass binary X-ray sources were interpreted using the two-component model where the soft, ~ 1 keV, component originates from the inner accretion disk while the hard, ~ 2 keV, component comes from the boundary layer formed between the accretion disk and the surface of the neutron star⁴. These observational facts have confirmed and verified accretion disks in various astrophysical objects. To investigate accretion disks further, I propose a strategy to search for and detect the peculiar properties of the emergent radiation from the inner region of a relativistic accretion disk around a compact object, that is, the asymmetric feature of light curves during the eclipse of the disk.

In the vicinity of compact objects such as neutron stars or black holes, the rotating velocity of accretion disks is of the order of the speed of light⁵. Hence, the radiation originating from the disk suffers from a significant Doppler effect and is shifted towards blue or red depending mainly on whether the radiating part of the disk is approaching the observer or not⁶. Due to this Doppler effect, the flux of the approaching part can greatly exceed that of the receding part. Therefore the accretion disk has an asymmetric appearance⁷. By use of X-ray facilities with sufficient sensitivity and time resolution, this asymmetric feature can be observed when an eclipse of the inner part of accretion disks occurs. Thus, here I first estimate the variation of the observed flux predicted by the simple model and discuss the possibility of observation in the near future.

Suppose a disk of radius R surrounds a compact object of mass M in a binary system. The disk is assumed to have a uniform surface brightness B_0 for simplicity. The emission from the central object is neglected, or the disk is considered as surrounding a black hole. Moreover, the disk is assumed to be inclined to the observer and rotating anticlockwise. That is, the left semicircle of the disk is approaching the observer and blueshifted, while the right is receding and redshifted. Finally, I assume that the brightness B_- of the blueshifted part is enhanced as $B_- = B_0/(1+z_{\min})^4$, whereas the brightness B_+ of the redshift part is reduced by $B_+ = B_0/(1+z_{\max})^4$. Here, $z_{\min}$ and $z_{\max}$ are, respectively, the minimum and maximum redshift of the radiation from the disk. For values of $z_{\min}$ and $z_{\max}$, I take $1/(1+z_{\min}) = 1.2$ and $1/(1+z_{\max}) = 0.8$ (ref. 6). This leads to $B_+/B_- = 0.198$.

As the radiation from the disk is emitted mainly in the inner part of the disk and the redshift also becomes minimum or

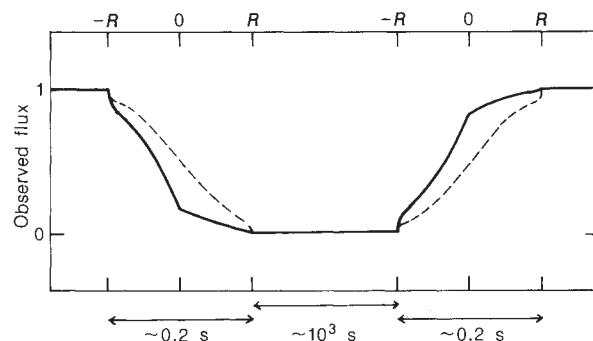


Fig. 1 The light curve predicted by the simple model. The solid curve represents the light curve of the relativistic disk, while the dashed one denotes that of the non-relativistic one. The former has a prominent feature: asymmetry.

maximum in the same region^{6,7}, this simple model may represent the essential properties of the realistic accretion disk.

How are the light curves affected by the eclipse of the disk by its companion? To calculate the light curve, I assume that the disk rotates in the same sense as the companion moves in its orbit. The light curve predicted by the present simple model is shown in Fig. 1 by a thick curve. In contrast to the non-relativistic case where the Doppler effect is not considered and the disk radiates with uniform surface brightness (denoted by a dashed curve), the light curve of the relativistic disk is not symmetric before and after the total eclipse. This is the most prominent feature of the accretion disk rotating with relativistic speed.

This asymmetry is easily understood as follows. During the early stage of the eclipse, the light curve will drop quickly because the left blueshifted part of the disk is first occulted by the companion. This area is then covered and we receive only the radiation originating from the receding part. Accordingly, the light curve gradually drops. After the end of the total eclipse, the situation is reversed; the approaching part appears first and the receding part next. Can we detect this asymmetric feature inherent in the relativistic disk?

Now I briefly estimate the timescale of the variation of light curves via eclipse and compare it with the performance of the recent facility. If the eclipse of the disk takes place through the companion of mass m and radius r_* , the eclipsing timescale is $\Delta t \sim 2R/v$, where v is the orbital velocity and $v = (G(M+m)/a)^{1/2}$, a being the orbital separation. This rough estimate gives

$$\Delta t = 0.128 (R/3r_g)(a/R_\odot)^{1/2}(M/10 M_\odot) \times ((M+m)/10 M_\odot)^{-1/2} \text{ s}$$

where r_g is the Schwarzschild radius of the compact star. In addition, the duration time of the total eclipse of the disk is $t_{\text{tot}} = 2r_*/v = 10^3 (r_*/R_\odot)(a/R_\odot)^{1/2}((M+m)/10 M_\odot)^{-1/2} \text{ s}$.

The Japanese third X-ray satellite, Astro-C, was launched on 5 February 1987 and named Ginga (galaxy). Several X-ray facilities are on board. Of these, the large-area proportional counter (LAC), the main facility, has a sensitivity in the range 1.5-30 keV and a maximum time resolution of 0.98 ms (ref. 8). Hence, the high time resolution of Ginga will easily detect the light curve asymmetry of the accretion disk around a compact object discussed here, because the radiation from the inner region of the accretion disk itself has already caught up with the X-ray satellite Tenma⁴.

The detection of such an effect of the light curve asymmetry has several astrophysical meanings. One is an investigation of the accretion disk model around the compact object. In particular, we can investigate in detail the inner disk region which has been theoretically suggested to be unstable⁹. Another is the

direct verification of the relativistic effect which causes the asymmetric feature. That is, observations of the radiation originating from the inner part of the accretion disk will give the information on space-time structure, for instance, whether a black hole rotates or not.

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The 152-day periodicity of the solar flare occurrence rate

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The occurrence rate of solar flares exhibits a periodicity of about 152 days. This periodicity was discovered from analyses of flares observed with the Solar Maximum Mission (SMM), and confirmed by analysis of flares observed by other observatories. This periodicity was also discovered from the flare rate of solar cycle 20 (1965-75). The cause of the 152-day periodicity still remains a mystery. But answers to the following questions will enhance our understanding of it. (1) Is the periodicity a local or a global phenomenon? (2) Is the periodicity due to the interaction of 'hotspots' rotating at different rates such that they align with one another once every 152-day period? (3) Is the periodicity due to the interaction of rotating features originating from g-mode oscillations? Here we report our analysis of 'major flares' observed with the Hard X-ray Burst Spectrometer (HXRBS) aboard SMM and we conclude that the 152-day periodicity is a global phenomenon, and that the answers to questions (2) and (3) are negative.

Rieger *et al.*¹ discovered the 152-day periodicity by analysing flares detected by the Gamma-Ray Spectrometer (GRS) aboard SMM during the interval from February 1980 to August 1983. They also confirmed this periodicity by analysing flares with HXRBS peak count rates above 1,000 counts s⁻¹ and by analysing flares with energy fluxes greater than 2.5×10^{-5} W m⁻² in 1-8 Å soft X rays, measured with Geostationary Operational Environmental Satellites (GOES), for the same time interval. In addition, Kiplinger *et al.*² analysed ~7,000 flares observed with HXRBS during February 1980 to September 1984, and found a periodicity of 158 days (see also ref. 3).

The time interval covered by Rieger *et al.*¹ corresponds to about eight periods of 152 days, and all eight peaks are found in the flare occurrence rate. But if we examine the flare rate after this interval, the peak expected in October 1983 is missing, and the next peak expected in March 1984 is also missing, but very active flare production was observed instead in April and May 1984^{4,5}. Therefore, it became important to determine whether this periodicity was a transient phenomenon. To answer this question, Bogart and T.B.⁶ analysed flares producing microwaves with peak flux densities greater than 10 solar flux units (s.f.u. = 10^{-22} W m⁻² s⁻¹) measured at frequencies above 1 GHz, for the time interval from April 1966 to December 1983. They found this periodicity not only in solar cycle 20 but also found that the periodicity was phase coherent through cycles 20 and 21. By analysing H α flares observed during the period from 1964 to 1983, Ichimoto *et al.*⁷ also found the periodicity from cycles 20 and 21.

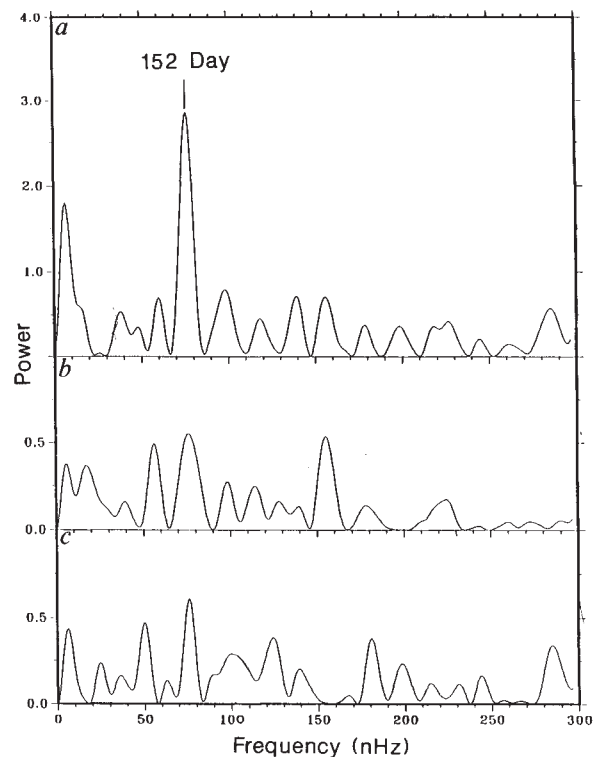


Fig. 1 Fourier power spectra for major flares observed with HXRBS from February 1980 to December 1983: *a*, all flares, *b*, northern hemisphere flares, and *c*, southern hemisphere flares. In *b* and *c* there exist some other peaks comparable to the 152-day peaks, but only the 152-day peaks are common to both hemispheres.

To study the distribution of flares on the Sun, one has to determine the appropriate rotation period of the Sun. T.B.⁵ analysed the coordinates of 'major flares,' whose hard X-ray peak intensities were above 1,000 counts s⁻¹ as measured by HXRBS aboard SMM, for the interval February 1980 to August 1985. T.B. showed that active zones exist where flare activity is much higher than other places, and that the active zones rotate with a synodic period of 26.75 days. (All the rotation periods in this paper are synodic, unless otherwise specified.) From a stack plot of the equatorial zone of magnetic-polarity synoptic charts, one finds that large-scale magnetic polarity patterns of the Sun show a rotation with a period similar to this. Also from a stack plot of magnetograms one finds long-lasting magnetic features rotating at a similar rate⁷. From these facts, T.B.⁵ proposed that subsurface sources of activity rotate with a period of about 26.75 days.

The 'active zones' of ref. 5 are similar in concept to 'active longitudes' or 'preferred longitudes' proposed earlier⁷, but they are different in the following aspects. First, active zones of the northern hemisphere are not in the same longitude bands as those of the southern hemisphere. Second, active zones are found to rotate faster than the Carrington rate which was assumed to be the rotation rate of active longitudes.

Because the locations of the HXRBS major flares have already been identified from coincident H α observations, we use the HXRBS major flares as our database. We used daily numbers of major flares as a time series for Fourier analyses. Figure 1 shows Fourier power spectra for 442 major flares observed during the period from February 1980 to December 1983. The most prominent peak is found at 76 nHz (152 days) in Fig. 1*a* for all the flares, and the same peak is observed from the power spectra calculated for the northern and southern hemisphere flares taken separately.

The values of the periodicity determined by various authors are as follows: 154 days for GRS flares and 152 days for flares

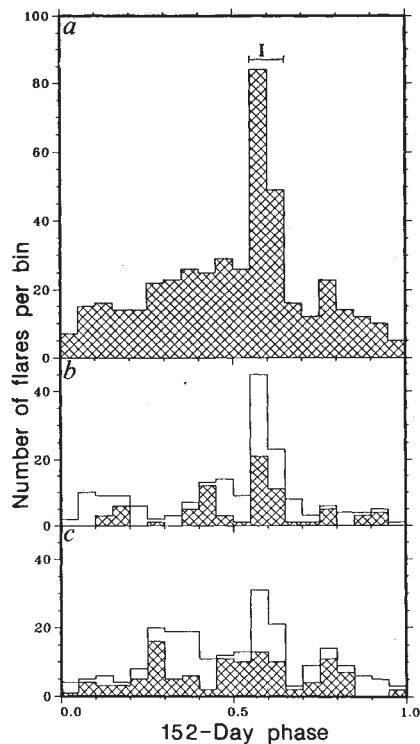


Fig. 2 Number of flares as a function of 152-day phase. Phase 0 point is 00:00 UT on 15 March 1980. *a* (For all flares), shows an impulsive increase of flare activity in phase interval *I*. The cross-hatched area in *b* (northern hemisphere flares) represents flares observed in the prominent active region (216–246 degree longitude interval) of the northern hemisphere, and the cross-hatched area in *c* (southern hemisphere flares) represents flares observed in the southern hemisphere active zones (60–150 and 260–320 degree longitude intervals). See ref. 5 for details of active zones.

with GOES class above M2.5 (ref. 1); 151.8 ± 1 days for microwave flares for solar cycles 20 and 21 (ref. 6); 155 days for cycle 20 and 153 days for cycle 21 for H α flares⁷; 158 days for HXRBS flares^{2,3}. The periods determined for cycle 21 are all in the 152–154 day range, except for the value of Kiplinger *et al.*². They obtained a larger value, because they included flares observed in 1984 in their analysis and a strong flare activity in 1984 April was about 40 days later than that expected from the 152-day periodicity. If we include flares observed in 1984 and 1985, the position of the peak shifts to 158 days, consistent with Kiplinger *et al.*², but the peak value of the power spectrum decreases. From both the autocorrelation function of the flare rate and the variance of the phase diagram for the 1980–1985 interval, we confirm that 152 days is the best period, consistent with Bogart and T.B.'s result⁶ which has the best spectral resolution. After May 1983, no significantly strong flare activity was observed at times expected from the 152-day periodicity. Similarly, during the decay phase of cycle 20, spells of strong flare activity (for example, August 1972; July and September 1974) were observed outside the peak phase of the 152-day periodicity⁶. Therefore, we think it appropriate to analyse flares observed before 1984 in studying the 152-day periodicity.

The error of the power estimation due to count statistics is of the same order as the noise at very high frequencies¹⁰, and for the result of Fig. 1*a* it is only about 0.03. Estimation of the statistical significance of the 152-day periodicity is difficult, because flare occurrence times are not all independent as the occurrence times of flares from the same active regions are close together. To estimate the statistical significance, we have performed Monte Carlo simulations by randomizing flare occurrence times with the following two restrictions. First, the solar cycle dependence of the flare occurrence rate follows the 5-

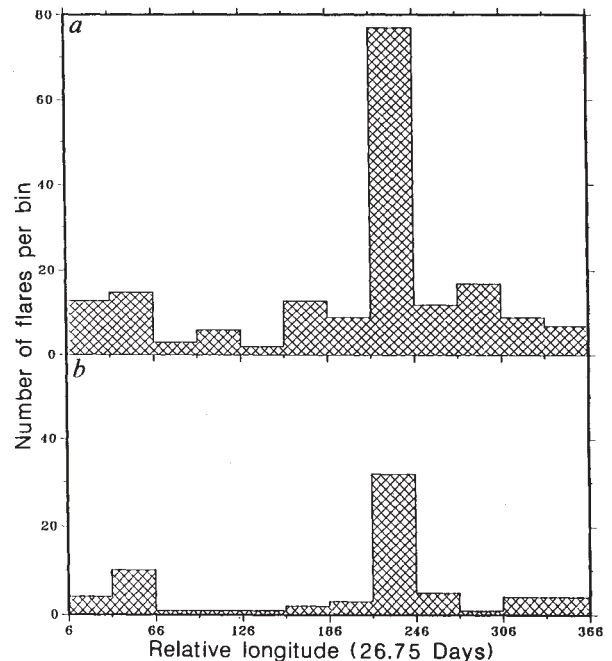


Fig. 3 Northern hemisphere flare distribution as a function of relative longitude. In calculating the relative longitude, we take the synodic rotation period to be 26.75 days and the Eastern limb at 00:00 UT on January 1980 to be the zero longitude (see ref. 5). *a*, All northern hemisphere major flares for 1980–83; *b*, flares that occurred during the peak of the 152-day periodicity (phase interval *i* of Fig. 2).

month averages of the actual flare rates. Second, the relative timing of the flares from the same active regions are fixed according to the actual observations, although the absolute timing is randomized. From each simulated flare rate we calculate the Fourier power spectrum and find the maximum power density in the 50–200 day (58–231 nHz) interval. From the distribution of the maximum power obtained from many simulations one can estimate the statistical significance of a peak in the actual spectrum. For the whole disk case, we found the median value of the maximum power density for 1,000 simulations to be about 0.95 and the actual peak value 2.86 at 152 days (Fig. 1*a*) to be significant at about the 6σ level. Except for the 5-nHz peak due to the length of the data run and the 152-day peak, all the peaks of Fig. 1*a* are below 1, and therefore none of them is statistically significant. We find several peaks comparable to the 152-day peaks in Fig. 1*b* and *c*, but we consider only the 152-day periodicity to be statistically significant as this is the only periodicity that is common to both hemispheres (and whose phase is the same, as shown later). Monte Carlo results show that in about 10% of simulations peaks bigger than the observed 152-day peaks in Fig. 1*b* and *c* are found at random frequencies in the 58–232 nHz interval for the northern and southern hemispheres taken separately. But, the chance of getting such a large peak for both hemispheres taken together is very small.

Figure 2 shows the phase diagram for the 152-day period, where the point of zero phase was arbitrarily taken as 00:00 UT on 15 March 1980. We can see an impulsive increase of flare activity in the phase interval *I* (0.55–0.65) on top of a sinusoidal variation of the flare rate. Fig. 2*b* and *c* show the phase diagrams for the northern hemisphere and the southern hemisphere separately. In both cases, flares produced in the active zones are shown by cross-hatched areas.

Figure 3*a* shows the longitude distributions of all northern hemisphere flares in a system rotating with a 26.75-day period. We find a very prominent active zone in the 216–246 degree

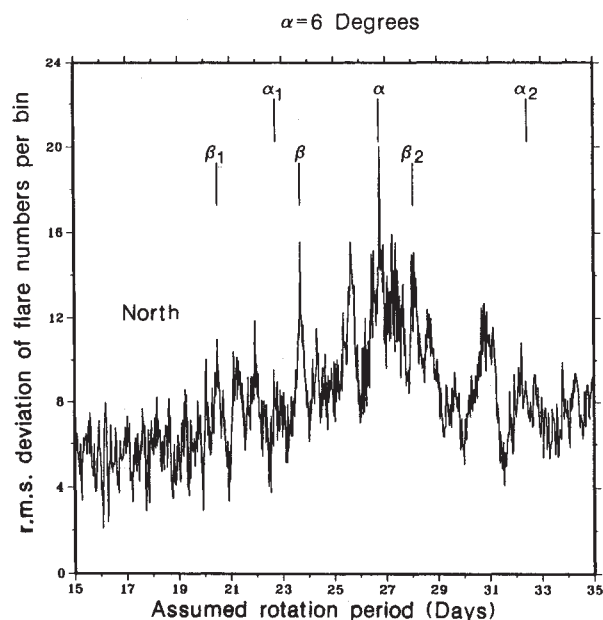


Fig. 4 r.m.s. Deviation of flare numbers per longitude bin as a function of assumed rotation period. For the 1980-83 period. Peak α is at 26.75 days, and peak β is at 23.69 days. Periods which satisfy equation 1 for $P_1 = 26.75$ days are indicated by α_1 and α_2 . Periods which satisfy equation 1 for $P_1 = 23.69$ days are indicated by β_1 and β_2 . Notice that peaks at 17.22 and 19.37 days, expected on the basis of Wolff's proposal for $S = 1$, are missing.

longitude interval. Figure 3b shows the longitude distribution of northern hemisphere flares produced in the phase interval I of the 152-day period (see Fig. 2). The longitude distributions shown in both panels of Fig. 3 are similar to one another. About 45% of flares are found in the prominent active zone in both cases. Similar results are observed from analysis of southern hemisphere flares.

We can deduce the following from Figs 1-3. First, both northern hemisphere flares and southern hemisphere flares show the 152-day periodicity independently. Second, the phase is coherent between the two hemispheres. Third, flares produced within the active zones as well as flares produced outside the active zones exhibit the periodicity (see Fig. 2). From these facts we conclude that the 152-day periodicity is a global phenomenon, suggesting that the underlying cause of the periodicity is a mechanism involving the whole Sun.

This conclusion is different from one of the conclusions of Ichimoto *et al.*⁷ that the 152-day periodicity originates from strong 'magnetized streams' appearing in stack plots of synoptic magnetic charts. The Carrington longitudes of the streams increase with time, and one can estimate the rotation period of these streams to be about 26.75 days. The magnetized streams are equivalent to the active zones of T.B.⁵. Flares produced in the active zones do indeed exhibit the periodicity, but so do flares produced outside the active zones.

Let us now address the second question posed. To produce the 152-day periodicity, the rotation periods of active zones (hot spots) should satisfy the following relationship

$$1/P_1 - 1/P_2 = \pm 1/152 \quad (1)$$

where P_1 and P_2 are rotation periods in units of days; or equivalently

$$\omega_1 - \omega_2 = \pm 76 \text{ nHz} \quad (1a)$$

where ω_1 and ω_2 are rotation frequencies.

To search for hotspots rotating at undetermined rates, one can calculate the r.m.s. deviations of flare numbers per longitude bin as a function of assumed rotation rate. If flares are concentrated in one or two bins in a system rotating with a certain

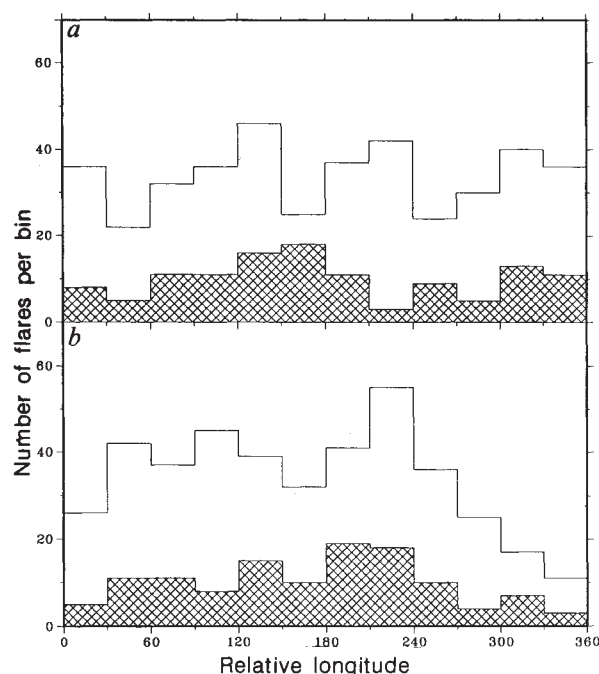


Fig. 5 Longitude distributions of flares in systems rotating with periods of 19.37 days (a) and 36.14 days (b). Hatched areas indicate flares produced during the peak phase (interval I in Fig. 2) of the 152-day periodicity, and outside histograms indicate all the flares with known coordinates. According to Wolff's model, we expect to see flare concentration in a system rotating with a period of 19.37 days ($S = 1$, $l = 2$ case) or in a system rotating with a 40.22 days ($S = 2$, $l = 3$ case).

period, as in Fig. 3, the r.m.s. deviation will be large for that period. Figure 4 shows the r.m.s. deviations of flare numbers per bin as a function of assumed rotation period for the northern hemisphere. We can see a prominent peak at 26.75 days, which is caused by a centre of subsurface activity rotating with this period⁵. For $P_1 = 26.75$ days, $P_2 = 22.75$ days or 32.46 days satisfies equation (1). However, there is no peak at either 22.75 days or at 32.46 days, suggesting active zones (or hot spots) rotating with such periods. Furthermore, as we have seen, the periodicity is not due mainly to flares produced in active zones rotating with a period of 26.75 days. Therefore, we conclude that the 152-day periodicity is not due to interactions of active zones rotating with a 26.75-day period and active centres rotating with different periods.

We can see a peak also at a 23.69-day period for the northern hemisphere, and there is a peak at a 23.86-day period for the southern hemisphere (Fig. 7 of ref. 5). It is not yet clear whether such peaks are chance happenings or represent real hotspots rotating with a 23.69-day period. The pair of periods 23.86 days and 28.30 days satisfy equation (1), but there is no evidence of a hotspot rotating with a 28.30-day period in the southern hemisphere. For the northern hemisphere, there is a peak at about 28.06 days (β_2 of Fig. 4), which satisfies equation (1) with $P_1 = 23.69$ days. But, even if we take a system rotating with a 23.69-day period, the 152-day periodicity appears to be a global phenomenon. From the above arguments we conclude that the answer to the second question is negative.

Wolff^{11,12} has proposed that various g-modes of stellar oscillation with the same spherical harmonic index, l , can couple to form 'active bands' inside the Sun, in which energy generation is enhanced (Fig. 1 of ref. 11). These active bands rotate at the rate

$$\omega_l = \omega_r \left[1 - \frac{1}{l(l+1)} \right] \quad (2)$$

where ω_r is the rotation rate of the stellar layers in which g-modes

are concentrated. Wolff^{11,12} proposed that such patterns of active bands produce similar global patterns of low-velocity flow. When such active bands due to different l modes overlap, according to Wolff, global flow patterns are enhanced, and strong magnetic fields are generated in the longitudinal intervals where active bands overlap (Fig. 6 of ref. 11). The active bands due to different l modes overlap at a rate $S(\omega_l - \omega_r)$, where S is a symmetry factor (1 or 2). Thus the frequency of alignment is expressed by

$$S(\omega_l - \omega_r) = S\omega_r \left[\frac{1}{l'(l'+1)} - \frac{1}{l(l+1)} \right] \quad (3)$$

By Fourier analysis, Wolff¹² obtained a multi-peaked spectrum of the Zurich sunspot number for the interval 1749–1979. By choosing the only free parameter to be

$$S\omega_r = 762 \text{ nHz} \quad (4)$$

he was able to fit many of the peaks in the 70–130 nHz interval apparently corresponding to interactions of the $l=2$ set and higher harmonics. For this choice of $S\omega_r$, $S\omega_l$ becomes 635.0 nHz for $l=2$ and 698.5 nHz for $l=3$. To fit the data, Wolff¹² chose 629.34 nHz for $l=2$ and 703.83 nHz for $l=3$. The beat frequency between these two frequencies is 74.5 nHz. Not only did he fit a peak at 74.5 nHz but he also fitted peaks for $l=2$ and 4, $l=2$ and 5 and so on (see Fig. 4 of ref. 12). Because this frequency of 74.5 nHz is very close to that of the 152-day periodicity of the flare rate, Rieger *et al.*¹ proposed that this might be the cause of the 152-day periodicity.

According to Wolff's model, when the active bands of an $l=2$ mode and those of an $l=3$ mode overlap and interact, flare production will be enhanced. This enhanced flare activity is expected to occur in the longitude bands where active bands of the two l modes overlap (see Fig. 6 of ref. 11). Following Wolff's model, the peak phase of the 152-day periodicity corresponds to time intervals when the active bands of the $l=2$ mode and those of the $l=3$ mode overlap and interact. Because the active bands of the $l=2$ mode rotate at a rate of ω_2 , in a system rotating at this rate the active bands are always in the same longitude intervals. Therefore, the flares observed during the peak phase of the 152-day periodicity (interval I in Fig. 2) are expected to be concentrated mainly in certain longitude bands in a system rotating at ω_2 . The same is true for a system rotating at ω_3 .

We can, therefore, test Wolff's model by investigating longitude distributions of the flares produced during the peak phase of the 152-day period in systems rotating at the rates of ω_2 and ω_3 . If $S=1$, one active band of the $l=2$ mode rotates at a rate of 629.3 nHz, which corresponds to 19.37-day synodic rotation, and an active band of the $l=3$ mode rotates at a rate of 703.8 nHz, which corresponds to 17.22-day synodic rotation. Therefore, if Wolff's model is correct and $S=1$, we expect the flares observed during the peak phase of the 152-day periodicity to be concentrated in a certain longitude band in a system rotating with a 19.37-day synodic period. Similarly, we expect to see flare concentration in a certain longitude band in a system rotating with a 17.22-day synodic period. If $S=2$, we expect two active bands for each mode, and the rotation frequencies are halved. The corresponding synodic rotation periods are 40.99 days for $l=2$ and 36.14 days for $l=3$. For this case, we expect that flares observed in the peak phase of the 152-day periodicity will be mainly concentrated in two longitude bands $\sim 180^\circ$ apart in both systems rotating with periods of 36.14 days and 40.90 days. In Fig. 5 we plot longitude distributions of flares (both from northern and southern hemispheres) for systems rotating with periods of 19.37 days ($S=1$, $l=2$ case) and 36.14 days ($S=2$, $l=3$ case). Hatched areas indicate the flares observed during the peak phase of the 152-day period. We do not find any pronounced concentrations of flares in longitude. We have also performed a similar analysis for periods of 17.22 days and 40.90 days, and failed to find any pronounced concentration of

flares in longitude. Therefore, we conclude that the beat between g -mode solar oscillations of $l=2$ and $l=3$ is not the cause of the 152-day periodicity. Consistently with this result, we fail to find peaks corresponding to beat frequencies of different l numbers in the Fourier spectrum of all HXRBS flares³ or in the power spectra of flare occurrence rates analysed by others^{1,6}. We have also shown earlier that only the 152-day periodicity is statistically significant.

This research was supported by NASA and ONR at Stanford University. The main database of this work is the major flares observed with HXRBS. We thank Brian Dennis for making the data available. We benefited from discussions with Rick Bogart, Harald Henning, Todd Hoeksema, and Phil Scherrer. We thank Dr James Lemen for giving helpful comments in the review process.

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Zero electrical resistance at 106 K in $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$

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The phenomenon of high-temperature superconductivity in oxides has made rapid strides since the reports of possible high- T_c superconductivity in the La–Ba–Cu–O system¹. The occurrence of superconductivity above 90 K was first reported by Wu *et al.*² in a multi-phase sample with the nominal composition $\text{Y}_{1.2}\text{Ba}_{0.8}\text{CuO}_{4-\delta}$. Since then it has been demonstrated that the superconductivity in this material originates from a single minority phase $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ with a well-defined stoichiometry^{3,4}. The structure of this phase has been determined by high-resolution neutron powder diffraction (refs 5, 6 and refs therein) to be orthorhombic, with a tripled perovskite-like unit cell resulting from the ordering of Y and Ba cations and oxygen defects. Here we present the results of a study in which we have synthesized a single-phase $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ material and observed a zero-resistance state (T_c) up to as high as 106 K after sintering it in an oxygen atmosphere. Our results show that there is an optimum sintering time, beyond which the superconducting properties deteriorate.

The material $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ was prepared by standard powder-metallurgical techniques from pure Y_2O_3 , BaCO_3 and CuO powders. The mixtures were uniformly mixed, pelletized and sintered at 1,175 K for 72 h in air. The product was reground, pelletized and sintered in flowing oxygen for periods varying from 4 to 30 h at 1,225 K, and then slowly furnace-cooled. The temperature during sintering was maintained to within 1 K with a Stanton–Redcroft temperature controller-programmer (Model UTP). The X-ray powder diffraction data for samples thus prepared are in complete agreement with those of Cava *et al.*⁴, indicating that our samples are single-phase.

Electrical resistivity measurements were made using a standard four-probe method with sample dimensions $\sim 1 \text{ mm} \times$

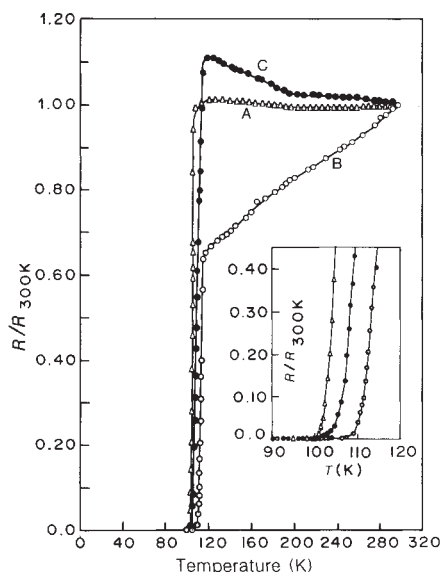


Fig. 1 Normalized electrical resistance plotted against temperature for $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$. Curves A, B and C are for samples sintered in oxygen at 1,225 K for 4, 18 and 30 hours, respectively. The inset depicts the fall to zero electrical resistance in greater detail.

1 mm $\times$ 10 mm. Fine copper wires applied with silver paint served as electrical leads and constant d.c. currents of 0.1 and 1.0 A cm^{-2} were applied during measurements. Temperature was measured with a calibrated copper-constantan thermocouple and the error in measurement of temperature did not exceed ± 0.5 K. The resistance at 300 K was in the range 300–400 $\mu\Omega$ cm. Figure 1 shows the normalized resistivity data for three representative samples of $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ which were sintered in flowing oxygen at 1,225 K for 4 h (A), 18 h (B) and 30 h (C), respectively. All three samples show the onset of superconductivity (T_{∞}) between 120 and 125 K, and the zero-resistance state (T_{cf}) occurs at 99.5, 106 and 101 K, respectively. The width of the transition, ΔT_{c} (that is, the temperature interval during which the resistance falls from 90% to 10% of the resistance at T_{∞}), is quite small: 3, 5 and 5 K, respectively, for A, B and C. On sintering in oxygen for up to 18 h (Fig. 1, sample B), the T_{cf} value improves to 106 K and the sample shows metallic-like behaviour at room temperature, but a further 12 h of sintering in oxygen (sample C) leads to a deterioration of the superconducting properties: T_{cf} falls to 101 K and the normal-state resistance shows semiconducting behaviour. In fact, we observed a further fall in T_{cf} to ~ 80 K with a further 16 h of sintering in oxygen (not shown in Fig. 1). These observations underline the role of oxygen stoichiometry, which can apparently be optimized for the best superconducting characteristics. The exact value of the oxygen content in our samples is not known and is being ascertained. We believe that T_{cf} in $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ may be improved to ~ 120 K, the onset temperature, by fine-tuning the oxygenation at a certain optimum sintering temperature, which will emerge from further experiments under way. Beno *et al.*⁷ have postulated that oxygen pressure during sintering can be a controlling factor. We have attempted to sinter our samples in a high oxygen pressure (~ 100 atm) at 1,200 K. The preliminary results, however, did not point to any improvement in superconductivity characteristics.

A measurement of the Planck constant

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The advent of the Josephson effects and the more recently discovered quantized Hall resistance¹ has led to rapid progress in the measurement of certain combinations of the fundamental constants, so much so that the realization of the electrical units has increasingly limited our knowledge of the value of many of the fundamental physical constants (E. R. Cohen and B. N. Taylor²). We deduce a value for the Planck constant of $6.626\,0688(37) \times 10^{-34}$ Js from the results of the National Physical Laboratory (NPL) quantum Hall and Josephson effect measurements, and the NPL moving coil realization of the electric watt. This value is comparable with the 1986 set of values recommended by CODATA, and so confirms our understanding of the physics of condensed matter and atomic spectroscopy. Its accuracy is capable of further improvement.

Taking the Josephson effect voltage to be E_J and the quantum Hall resistance to be R_H , and arranging a *gedanken* experiment of the type discussed by Kose and Woger³ such that E_J also equals the Hall voltage E_H and the associated current is i_H , one can easily see that there are four measurements which may be performed in terms of SI units rather than laboratory as-maintained units: (1) a measurement of E_J with an electrometer to obtain $2e/h$, (2) a measurement of R_H in terms of the calculable capacitor to give h/e^2 ; (3) an SI measurement of the current i_H with a current balance which would yield e ; and (4) a measurement of $W_H (= E_H \cdot i_H)$ with a moving coil apparatus, which yields h .

It is usual in these experiments to relate, as an intermediate step with negligible loss of accuracy, the Josephson effect voltage and the quantum Hall effect resistance to the laboratory maintained units of voltage and resistance, $V_{\text{LAB}} (= (K_V)_{\text{LAB}} V)$ and $\Omega_{\text{LAB}} (= (K_{\Omega})_{\text{LAB}} \Omega)$ respectively, where $K_V = (2e/h)_{\text{LAB}}/(2e/h)$ and $K_{\Omega} = R_H/(R_H)_{\text{LAB}} = (h/e^2)/(h/e^2)_{\text{LAB}}$.

Sloggett *et al.*⁴ have already discussed a measurement of $2e/h$ by their mercury pool electrometer method, and measurements relating to SI determinations of h/e^2 are discussed by E. R. Cohen and B. N. Taylor². The realizations of the ampère have not proved capable of refinement to sustain their relevance to the measurement of the electronic charge e and this aspect is also discussed by Cohen and Taylor in ref. 2. Here, we are concerned with the establishment of (4). The moving coil measurements of Kibble *et al.*⁶ have previously been used to realize the ampère and determine K_V (a preliminary value for $(K_V)_{\text{NPL}}$ was reported to the September 1986 meeting of the Comité Consultatif d'Electricité⁴ and an effect which had necessitated an expansion of the systematic uncertainty has been eliminated since then), for which purposes the results of a calculable capacitor determination have to be used. In fact the moving coil apparatus measures the NPL as-maintained watt in terms of the watt of the SI definition, $W_{\text{NPL}} (= (K_W)_{\text{NPL}} W)$. This allows an SI measurement of the Planck constant as:

$$(K_W)_{\text{NPL}} = (2e/h)_{\text{NPL}}^2 \cdot (h/e^2)_{\text{NPL}} \cdot h/4 \quad (1)$$

In the NPL moving coil apparatus described in ref. 6, a current i_{NPL} flows through a long rectangular coil suspended vertically from the arm of a precision balance. When the lower end is in a uniform horizontal flux density B , the mass on the scale-pan must be changed by m in order to sustain the balance condition. In a separate measurement the coil is moved vertically with a

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uniform velocity v inducing a voltage E_{NPL} . Then:

$$(K_W)_{\text{NPL}} = mgv / (E_{\text{NPL}} \cdot i_{\text{NPL}}) \quad (2)$$

where g is the acceleration due to gravity in m s^{-2} .

The mass m is 1 kg for a 3,362 turn coil carrying 10 mA in an applied flux density of 0.7 T and the repeatability of the balance is 30 μg . An induced e.m.f. of 1 V_{NPL} results from a uniform coil velocity of 2 mm s^{-1} . The ratio of induced e.m.f. to coil velocity is measured throughout the coil motion and, after fitting a parabola to the measurements, the value corresponding to passage through the position that the coil occupies for the weighing is then deduced to 0.03 p.p.m. The standard deviation of a single $(K_W)_{\text{NPL}}$ determination is 0.1 p.p.m. and the result is based on averages taken on 26 separate days over a period of six months. The assigned uncertainty is conservatively assessed as being one half of the total spread in the measurements. The acceleration due to gravity was transferred from the NPL reference site with a precision of 0.02 p.p.m. by means of a gravimeter. The electrical measurements may be transferred to NPL maintained standards on a daily basis with an accuracy of 0.05 p.p.m.

Denoting the NPL-maintained volt as $(K_V)_{\text{NPL}}$ volt; and the NPL-maintained ohm at a time t as $(K_\Omega)_{\text{NPL}}(t)$ ohm in order to take account of the small drift-rate (determined by the quantized Hall resistance measurements of Hartland *et al.*⁷ as being 0.069(77) p.p.m. per yr) of our resistance standards, the quantities measured at NPL give, on combining equations (1) and (2):

$$h = 4(K_W)_{\text{NPL}}(t_1) \cdot (K_\Omega)_{\text{NPL}}(t_1) / [(h/e^2)_{\text{NPL}}(t_2) \cdot (K_\Omega)_{\text{NPL}}(t_2) \cdot (2e/h)_{\text{NPL}}^2] \quad (3)$$

$(K_W)_{\text{NPL}}(t_1)$ is the result of the moving coil measurement performed at a mean time t_1 (1985.3), $(R_H)_{\text{NPL}}(t_2) \cdot (K_\Omega)_{\text{NPL}}(t_2)$ is the quantized Hall resistance measurement of (h/e^2) made at a mean time t_2 (1985.5) and, by definition,

$$(2e/h)_{\text{NPL}} = 483\,594.0 \text{ GHz } V_{\text{NPL}}^{-1}$$

Our measurements can be reduced to a common time of 1986.0, so that in equation (3) $t_1 = t_2$ and $(K_\Omega)_{\text{NPL}}(t_1)/(K_\Omega)_{\text{NPL}}(t_2) = 1$. The results⁷ for $(R_H)_{\text{NPL}}$ may be combined with the results, which we report here, of the NPL moving-coil experiment. These are:

$$(K_W)_{\text{NPL}}(1986.0) = 1 - 14.95(56) \times 10^{-6}$$

and

$$(R_H)_{\text{NPL}}(1986.0) = 25\,812.8375(10) \Omega_{\text{NPL}}$$

Substituting in equation (3) we obtain

$$h = 6.626\,0688(37) \times 10^{-34} \text{ Js}$$

without needing to refer to an SI ohm realization from a calculable capacitor. This may be compared with the 1986 recommended value² of $6.626\,0755(40) \times 10^{-34} \text{ Js}$ and is in excellent agreement with the value of $6.626\,0681(41) \times 10^{-34} \text{ Js}$ obtained by Sloggett *et al.*⁵. The latter value was obtained by combining methods (1) and (2), that is, a realization of the SI ohm was required in order to express h in Js. The Sloggett *et al.* result and the recommended values are slightly correlated since the preliminary mercury-pool electrometer measurement⁸ was included in the data evaluated by E. R. Cohen and B. N. Taylor².

Many of the parameters in the NPL watt realization are already observed at the 10^{-8} level of repeatability. Consequently, our method appears to be capable of further refinement to yield a tenfold reduction in the uncertainty of the value of h . This, given a similar advance in measurements by alternative methods, would provide a stringent test of whether the Josephson effects and quantized Hall resistance indeed combine to yield a value

for the Planck constant from solid-state physics which might be compared to parts in 10^8 with a value from other branches of physics.

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Charge effects on the coalescence of water drops in free fall

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It has long been recognized that charge can affect the results of collisions in streams of water drops^{1,2}, between drops and a flat water surface³, between two drop streams^{4–6}, and for drops fired at suspended drops^{7,8}. Here we present the first observations of the effect of charge on the coalescence of colliding water drops that were initially falling freely at terminal velocity. Other experiments using uncharged drops show large discrepancies between drop streams or suspended drop results and free fall, terminal velocity results^{9–11}. Therefore, it is probable that previous work on charge effects should not be directly applied to clouds. The present findings support this contention, because the charge required to induce complete coalescence differs from previous results for similar drop sizes and size ratios by an order of magnitude. The following new features of charged drop interactions were observed: a unique impact angle dividing the coalescence from the non-coalescence region that is independent of charge, a two order of magnitude range of relative charge between drop bounce and complete charge-induced coalescence, a wide range of charge where a satellite drop occurs with temporary coalescence at higher charge levels the beginning of charge-induced permanent coalescence at the most grazing collisions.

The range of charges used in this experiment coincide closely with charges observed in nature: ranging from $\sim 1.0 \times 10^{-16} \text{ C}$ for drops in developing cumulus clouds to $\sim 1.0 \times 10^{-12} \text{ C}$ for drops in thunderstorms¹². Observations were taken with eight large-drop positive charges and a constant small-drop positive charge for more than 2,500 interactions between a 340 μm and a 190 μm radius drop. These drop sizes are typical of drizzle drops or small precipitation drops. Charges of like sign were chosen because drizzle drops in clouds are likely to have the same polarity¹³.

A unique computer-controlled apparatus was developed to allow the study of repetitive isolated pairs of freely falling drops with independently controllable drop charge. The computer operated two drop generators, positioned the drop collisions in the camera field of view, and synchronized camera and strobe operations. Figure 1 shows the arrangement of the drop generators, cameras and lighting system. The drop generators used hollow cylindrical piezoelectric transducers that vibrated radially to induce capillary waves on a jet caused by forcing water through a pin hole. The jet broke up into a stream of uniformly sized drops. Most of the drops were highly charged by using a cylindrical electrode around the point of jet breakup. These drops were deflected into a gutter as they fell through a

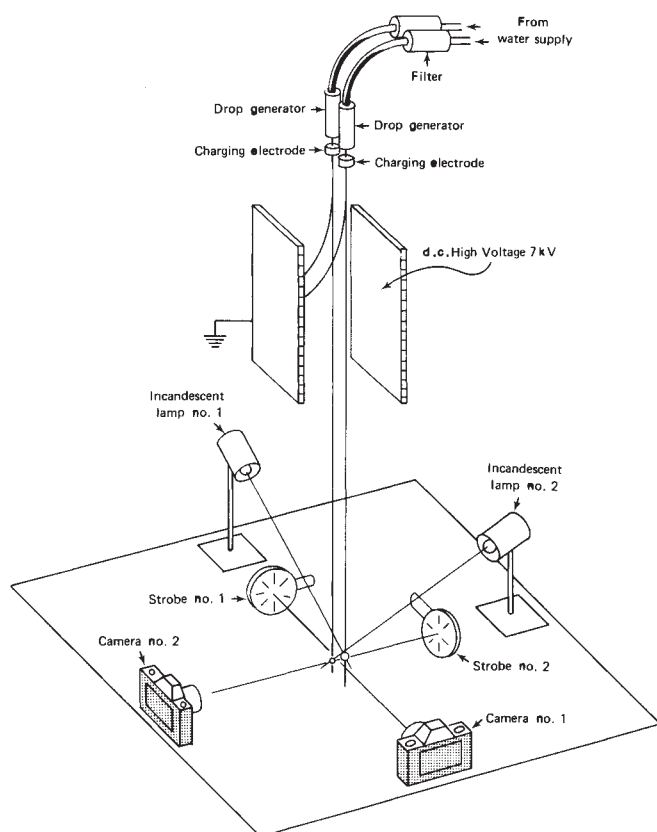


Fig. 1 The arrangement of major experimental apparatus components. The lines from the bottom of the drop generator and curving to the left illustrate the path of the highly charged drops that are deflected into a gutter and not used while the vertical lines show the path of the isolated drops to be studied.

strong horizontal electric field. A switching circuit controlled by the computer periodically imposed a pulse on the drop charging voltage. This produced individual drops with lower charge which fell through the horizontal electric field and into the experiment chamber. Micromanipulators attached to the drop generators were used to aim the drops to obtain an optimum number of collisions. The charge on the large and small drops was measured frequently during the observation period and adjustments were made to maintain desired drop charges.

Two cameras were used to record the collisions from orthogonal directions in a horizontal plane (Fig. 1). Incandescent lamps located behind and 30° above each camera's optical axis illuminated the falling drops. The camera's open shutter recorded streaks on the film as the drops passed. The impact angle for each collision was obtained from the distance separating the streaks just before impact. Size and drop shape information after collision was obtained from the back-lighted image of the drops on the film caused by a single low-intensity strobe flash.

The examples of streak signatures shown in Fig. 2 are from one orthogonal camera view. When the two drops do not collide the image in Fig. 2a resulted. Coalescence (Fig. 2b) occurred when two falling drops collided, made contact and united to form a single drop. Oscillation of the newly coalesced drop caused the waviness of the streak below the collision-coalescence point.

The air between the colliding drops must be expelled before the surfaces could make contact. Viscous forces opposed air film drainage and increased as the drop separation decreased. Draining of the air film was further retarded as the drops approached each other and their surfaces flattened. If the air

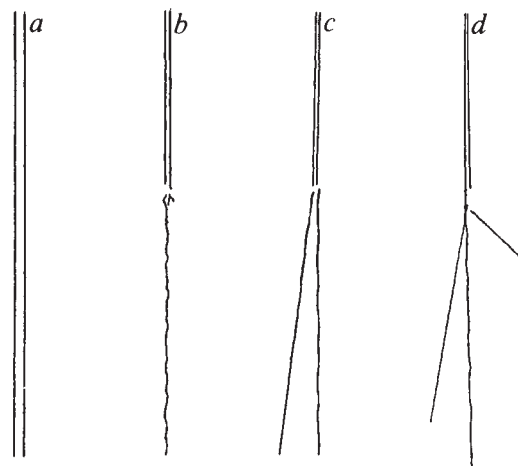


Fig. 2 Tracings of streak photography images: a, miss; b, coalescence; c, bounce; and d, temporary coalescence. Each image corresponds to a single frame of film. Examples without the strobe flash were chosen to illustrate better the streak images.

film was not depleted sufficiently to allow coalescence to begin during the time of interaction, the drops bounded apart (Fig. 2c).

Temporary coalescence (Fig. 2d) occurred when the drops coalesced and the resulting rotational energy was sufficient to pull them apart⁶. As the drops pulled apart, the filament of water connecting them was stretched. If this filament broke in two places, a satellite drop was formed. The short streak extending to the right in Fig. 2d was cast by a satellite drop. An average satellite radius of about $80\text{ }\mu\text{m}$ was measured on the strobe exposures and represents about 7% of the small drop mass.

Collision results are displayed in Fig. 3 as a function of impact angle and magnitude of difference in mean drop charge. The small drop charge, Q_r , averaged $2.5 \times 10^{-14}\text{ C}$ (with a standard deviation of $0.5 \times 10^{-14}\text{ C}$) while the large drop charge, Q_R , ranged from 2.0×10^{-15} to 2.1×10^{-12} . We observed an abrupt transition at a critical impact angle of $43^\circ \pm 1^\circ$ from a coalescence to a non-coalescence region. Collisions occurring for impact angles less than 43° always resulted in coalescence regardless of drop charge. For impact angles $>43^\circ$, the magnitude of the mean relative charge in Fig. 3 must exceed a value of $1.9 \times 10^{-14}\text{ C}$ ($Q_r = 2.1 \times 10^{-14}$ and $Q_R = 2.0 \times 10^{-15}$ for the experiment in which this minimum was observed) before charge influenced the result of collisions. Once a relative charge of about $3.0 \times 10^{-13}\text{ C}$ was exceeded bounce was completely suppressed and temporary coalescence resulted. Interestingly, the probability of satellite production decreased with further increases in relative charge. At higher charge, coalescence was induced when the relative charge exceeded about $8.0 \times 10^{-13}\text{ C}$. Figure 3 shows that coalescence begins at large impact angles (about 90°) and 'creeps in' as charge increases. Permanent coalescence at all impact angles resulted when the relative charge exceeds $2.0 \times 10^{-12}\text{ C}$.

The results in Fig. 3 show that coalescence is promoted by charge even though both drops carry positive charge. Electric field calculations¹⁴ show that when two spheres carrying the same polarity of net charge are close together the sphere with the largest charge can induce the opposite charge on the near surface of the other drop. For a size ratio of 0.5, the charge on the larger drop must exceed the net charge on the smaller drop by a factor of about three to induce an area of opposite charge. The electrical time constant for our drops was short enough to allow the induction process to polarize the near drop surfaces. Thus in the area of the trapped air film the oppositely charged drop surfaces enhanced film drainage by electrostatic attraction. Also, it has been proposed but not substantiated that an electrical

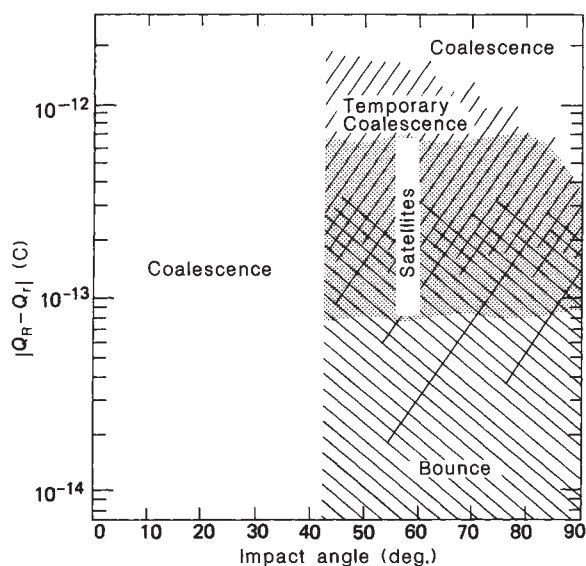


Fig. 3 Collision outcome as a function of impact angle and mean relative charge. The charge on the small drop was fixed at 2.5×10^{-14} C and the large drop charge varied from 1.5×10^{-15} to 2.1×10^{-12} C in eight irregular steps. Coalescence occurs in the clear area. The hatched area sloping down from left to right indicates the area where bouncing occurs. The hatched area sloping up from left to right delineates the region of temporary coalescence. The stippled area covers the drop charges and impact angles for which satellite production is likely if temporary coalescence occurs. The concentration of hatched area overlap suggests the probability of bounce and temporary coalescence in the transition region.

instability of the water surface may be responsible for charge-induced coalescence⁶. We have applied a theoretical treatment of the electrically induced instability of a flat liquid surface¹⁵ to the conditions between deforming drops¹⁶. A radius of deformation of half the radius of the smaller drop was deduced from our photographic evidence and a drop separation at the onset of coalescence⁸ of $0.1 \mu\text{m}$ was chosen. By restricting the instability wavenumber by the diameter of the deformation, the calculated charge required for instability was found to be in reasonable agreement with the observed transition between bounce and temporary coalescence.

Previous laboratory investigations using suspended drops or drop streams have determined threshold relative charges for complete coalescence that have ranged over three orders of magnitude. A charge of 3.0×10^{-14} C caused a stream of drops to coalesce with a flat water surface³. Drops in continuous streams have been induced to coalesce by charges of about 2.0×10^{-13} C (refs 4, 5) and 4.0×10^{-11} C (ref. 6). A charge of 3.0×10^{-12} C (ref. 7) and 3.0×10^{-11} C (ref. 8) caused coalescence in suspended drop experiments. The first value is close to ours, but their drop sizes and size ratios (0.07) are significantly different and thus comparisons are probably invalid. Charge-induced temporary coalescence has been reported^{3,4,8} but satellites have not. The wide range of charge required to induce coalescence in previous experiments ($3\text{--}4,000 \times 10^{-14}$ C) suggests that data on charge effects cannot be reliably extrapolated to drop interactions in clouds unless the conditions of the natural interactions are well-simulated. This conclusion is supported by measurements using uncharged drops at terminal velocity⁹⁻¹¹ in which results were found to differ significantly from drop stream and suspended drop experiments. The wide range of previous results from supported drops and drop streams and the ability to study drop interactions under free fall at terminal velocity give impetus to further research.

Horizontal electric fields have been shown to induce coalescence in bouncing streams of nearly equally sized drops aimed almost vertically⁵. It is unknown whether the long interaction times with favourable drop alignment are required for the field to induce coalescence. Clearly more experiments using isolated drop pairs falling at terminal velocity and colliding in vertical electric fields are needed.

Our new data demonstrate that the effects of charge on drop coalescence are significantly more complicated than had been thought previously. Several new phenomena were uncovered that remain unexplained. Of particular interest is the constant critical impact angle dividing permanent coalescence from bounce or temporary coalescence. Because drops colliding at angles $<43^\circ$ coalesce, it might be expected that drops colliding at angles just $>43^\circ$ almost coalesce. Using this reasoning, as charge increases, coalescence should be induced for these collisions first, and the critical angle should steadily increase with increasing charge until permanent coalescence finally occurs for the largest impact angle. Figure 3 shows this logic to be incorrect. In addition, Fig. 3 shows that the lowest charge-induced permanent coalescence occurs at the largest impact angles where the drops follow grazing trajectories. These collisions have the least interaction time and, after coalescence, the largest angular momentum which should be most conducive to temporary coalescence⁶. Another interesting aspect of our results is that satellites form at the intermediate relative charges required for temporary coalescence and are suppressed at higher relative charges. Although more refined experiments may help to explain these observations, an adequate understanding will probably require complex modelling of coalescing drops.

Our data indicate that the outcome of drop collisions varies considerably over the natural range of electrical environment found in clouds. Such variability suggests important consequences for precipitation evolution. Traditionally, for lack of better information, calculations of precipitation drop spectra have assumed that every collision results in coalescence¹⁷. Recent experimental results suggest that this assumption is invalid for weakly charged drops because many collisions result in bounce rather than coalescence¹¹. Comparisons between the computed evolution of precipitation using the traditional assumption of coalescence and a more realistic assumption including drop bounce, have shown significant differences in the concentration at which large precipitation drops are produced¹⁸. Thus we expect that a transition between bounce and coalescence induced by charge will significantly affect subsequent precipitation development. The observations presented here indicate that, in addition, moderate amounts of charge will induce satellites which, in turn, can serve as embryos for new precipitation drops. Thus the electrical state of the cloud can influence the subsequent evolution of precipitation.

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Mechanical properties of large quasicrystals in the Al–Cu–Li system

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Following the report of Dubost *et al.*¹, the synthesis and detailed growth characteristics of the quasicrystalline phase in the Al–Cu–Li system were recently documented². Here we have grown large (~3 mm) grains as well as orientated grains of the quasicrystals and have studied their mechanical properties. On account of the quasicrystals being brittle, we have employed successfully an indentation technique^{3–7}, normally used for brittle ceramics, to determine hardness and fracture toughness. Here we report the technique used and the values obtained.

In a previous study², we discussed in detail the scale and morphologies of crystals obtained at low- and high-growth rates during solidification of the T2 (Al₆CuLi₃) quasicrystalline phase in the Al–Cu–Li system. Faceted growth was observed to occur up to interface velocities of 6 $\mu\text{m s}^{-1}$, when alloys were directionally solidified under a temperature gradient of 10 K m^{-1} . For the present study, an alloy of bulk composition (Al 66 wt%, Cu 27.9 wt% and Li 6.1 wt%) was melted and directionally solidified at 1 $\mu\text{m s}^{-1}$, 27 $\mu\text{m s}^{-1}$ and 54 $\mu\text{m s}^{-1}$ under a gradient of 10 K m^{-1} . As reported earlier², the quasicrystals were faceted and randomly orientated at low-growth speed and became progressively finer and well orientated with an increase in growth speed. The final ingot was a cylinder, 60 mm in length and 10 mm in diameter with heat withdrawal being along the length of the sample. This was sectioned both along the length (heat withdrawal direction) and along a plane normal to the heat withdrawal direction. X-ray and mechanical property measurements were performed on the exposed quasicrystalline grains. Figure 1 shows five side branches of the quasicrystalline dendrite formed in the sample grown at 54 $\mu\text{m s}^{-1}$. The fivefold axis aligned itself with the flow direction for this sample². Grains were ~3 mm in size for the 1 $\mu\text{m s}^{-1}$ sample and became progressively smaller in samples made at higher-growth velocities². The intercellular region contained the R (Al₅CuLi₃), T1 (Al₂CuLi) and supersaturated aluminium phases. Details of the phase identification, size and alignments are given in ref. 2. X-ray diffractometer traces confirmed the large grains to be quasicrystalline.

Although 3-mm grains are large, they are still quite small for using conventional destructive techniques to measure mechanical properties. The presence of the intercellular material also hampers reliability of information from non-destructive (for example, ultrasonic) techniques. We have, therefore,

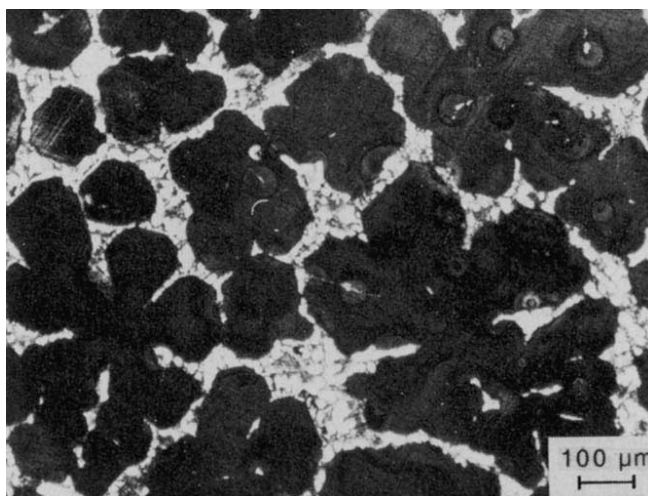


Fig. 1 Growth morphology of T2 dendrites grown at 54 $\mu\text{m s}^{-1}$. Section normal to growth axis.

employed the indentation methods described below. The region of indentation (~50 μm) was about two orders smaller than the grain, thus ensuring that the measured values reflect properties of the quasicrystals only. Initially the modulus/hardness ratio was calculated which was then used to calculate other properties.

Experimental determination of the H/E ratio (E is the modulus and H is hardness in GPa) was carried out following the procedure suggested by Marshall *et al.*³ incorporating a Knoop hardness indenter. The method is based on the understanding that during the unloading cycle of the indentation test, elastic recovery takes place in the samples being indented. The recovery that occurs is dependent on the H/E ratio of the material as formulated by Lawn and Howes⁴ who established this by measuring elastic recovery of materials when indented with a Vicker's indenter. But with a Vicker's indenter precise measurements were difficult because recovery is along the depth axis. Marshall *et al.*³ showed that the method can be simplified by using a Knoop indenter, where the elastic recovery takes place in the direction of the shorter diagonal and on the surface of the sample. The longer diagonal remains relatively unaltered. The diagonals can be measured optically, making the technique easy to use and reliable.

If the long and short diagonals of the Knoop indenter are denoted by a and b and those of the relaxed material are b' and a' , it can be shown³ that

$$b/a' = b/a - \alpha H/E \quad (1)$$

where α is a constant. By conducting tests on a number of ceramic materials with a wide range of H and E values, Marshall *et al.*³ obtained an experimental value of 0.45 for α .

Tests were carried out using a microhardness tester with eye-pieces fitted with crosswires. The eyepiece crosswires were in turn connected to a digital display for accurate measurement of lengths. Indentations were made with loads varying from 10 g to 100 g. The T2 grains were seen to crack easily. Often cracks overlapped the corners of indentations so that there was some difficulty in accurately measuring the diagonals. However, as pointed out by Marshall *et al.*³ the analysis of equation (1) is valid even when cracking takes place round the indentation. For the faceted grains (which grew at 1 $\mu\text{m s}^{-1}$) the measured H/E ratio varied somewhat with an average value of 0.018 ± 0.008 . The average Vicker's hardness value was measured as 3.82 ± 0.16 GPa. This gives a large scatter in the calculated modulus values and reliable values of the modulus could not be obtained by this method. Although the H/E values calculated above reflect properties of the quasicrystalline phase, a value of the modulus for the composite material (consisting of T1,

Table 1 Measured and calculated mechanical properties

Experimental conditions	H (GPa)	K_{IC} ($\text{MPa m}^{1/2}$)
From faceted crystals grown at 1 $\mu\text{m s}^{-1}$ imposed growth velocity	3.82 ± 0.16	1.023 ± 0.11
From some faceted and mostly unfaceted crystals obtained at 27 $\mu\text{m s}^{-1}$ imposed growth velocity. Plane of test was normal to heat flow direction	3.73 ± 0.27	2.31 ± 0.17
From unfaceted and aligned crystals obtained at 54 $\mu\text{m s}^{-1}$ growth velocity. Dendrites aligned with heat flow direction. Plane of test was normal heat flow direction	3.41 ± 0.29	5.16 ± 0.45

T2, R and the supersaturated aluminium) was also obtained. The density of the composite material was measured as $2,520 \text{ kg m}^{-3}$ and the corresponding ultrasonic velocity was $6,940 \text{ m s}^{-1}$. From these, the average modulus was calculated as 81 GPa which is similar to Li-containing Al alloys.

Fracture toughness, a measure of the material to resist cracking is denoted by K_{IC} (the plane strain fracture toughness). This was determined again by an indentation technique⁵. For fracture toughness measurements, a Vicker's indenter was used. K_{IC} values were obtained by measuring the crack lengths emanating from the corners of the indentation, the sizes of the diagonals of the indentation and a knowledge of the H/E ratio of the material calculated above. The original method, due to Evans and Charles⁵, was developed to obtain K_{IC} values of ceramics. This method is now routinely used for ceramic materials although there is some controversy regarding the crack geometry. The Evans-Charles⁵ method is based on the assumption that the crack beneath the indenter is median in nature, that is, the indentation together with the cracks emanating from its corners are parts of one large crack extending into the material. This assumption is valid only when the load ranges used are high and when the crack is fully developed. At low-load values, the crack is not fully developed. The cracks that generate at the corners of the indentation are separate individual cracks. This geometry is known as Palmqvist crack. Diagrams depicting the difference in geometry have been described by Niihara *et al.*⁶. A detailed analysis of the Palmqvist geometry is given in refs 6 and 7. With Palmqvist cracks the ratio of the crack to the indentation half-diagonal must be less than 2 (ref. 6). Here, this ratio was measured as <2 for a 50-g load. Accordingly, we have used the equation proposed by Niihara *et al.*^{6,7}

$$K_{IC} \phi / H \sqrt{a} (H/E \phi)^{0.4} = 0.035 (l/a)^{-1/2} \quad (2)$$

where K_{IC} is fracture toughness (in $\text{MPa } \sqrt{\text{m}}$), H is the hardness (in GPa), E is the elastic modulus (in GPa), l is the crack length, a is the indentation half-diagonal and ϕ is the constraint factor ($H/\sigma_y = 3$, where σ_y is the yield stress). Hardness was evaluated as

$$H = 0.464 P/a^2 \quad (3)$$

where P is the applied load. The H/E ratios calculated above were substituted in equation (2). The calculated value of toughness is $1.023 \pm 0.11 \text{ MPa m}^{1/2}$.

Mechanical properties were also measured on the surface normal to the heat flow direction for all three growth conditions. As shown in Fig. 1 and in ref. 2, the fivefold axis grew parallel to the heat withdrawal direction when the sample was solidified at rapid velocities. This became more pronounced with higher temperature gradients². Table 1 lists the property values obtained on all three samples.

We have presented above some mechanical properties for the quasicrystalline phase in the Al-Cu-Li system. The technique employed was the indentation method, the merits of which have also been discussed. Fracture toughness changes by five times with orientation. The fracture toughness values show that the material is brittle. The hardness was measured to be only a weak

function of orientation. On account of the material being extremely brittle, scatter was obtained in the calculated modulus values. Although a high modulus may be expected from the H/E values, the extreme brittleness of the material introduces a large scatter in the modulus values measured with the indentation technique. The modulus of the composite material (quasicrystalline, T1, R and aluminium phase) was measured to be 81 GPa. Typical values of modulus, hardness and toughness for a wide range of materials are given in Table 2. We note that in comparison with the materials listed in Table 2 the quasicrystal behaves like a material with high modulus, low toughness and medium strength, similar to the modulus approaching that of covalently bonded ceramics, the toughness of brittle glassy materials and the strength of metallic alloys.

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Acquisition of chemical remanent magnetization by synthetic iron oxide

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A fundamental assumption of palaeomagnetism is that rocks can provide a reliable record of the Earth's magnetic field. It is known that as magnetic minerals undergo chemical change they acquire a form of chemical remanent magnetization (CRM)¹. Indeed, the magnetic phases formed at different times in the history of the rock may add several components of CRM to its net magnetization², so that a valid application of palaeomagnetism to the interpretation of geological history requires a knowledge of the different components of CRM and the time of their acquisition. There are two mechanisms responsible for CRM³: mineral alteration and crystal growth. To understand the properties and acquisition mechanisms of CRM associated with the growth of mineral crystals, which is known as grain-growth CRM⁴, we have developed a technique for the synthesis of haematite crystals under controlled conditions. We find that the acquired magnetization is parallel to the applied magnetic field.

Previous experiments on the directional properties of CRM produced by the direct precipitation of a magnetic mineral from solution have been inconclusive³⁻⁷. Chemical remanence produced by the alteration of a magnetic parent mineral may be parallel to the field during alteration^{7,8}, may be parallel to the remanence direction of the parent^{9,10} or may be in some direction intermediate between the direction of the parent remanence and that of the field applied during alteration¹¹. Such apparent contradictions result from the control of remanence acquisition by several factors in addition to changes in mineral chemistry. These include magnetostatic and exchange interactions between parent and daughter minerals as well as changes in grain size and domain state during alteration¹². Well-controlled experiments are needed to isolate these factors.

To investigate the properties of grain-growth CRM, we have synthesized haematite from an aqueous ferric nitrate solution¹³

Table 2 Typical values of elastic modulus, hardness and toughness for various materials

Material	E (GPa)	H (GPa)	K_{IC} ($\text{MPa m}^{1/2}$)
Soda-lime Silica glass	70	5.5	0.74
Metallic glass ($\text{Fe}_{80}\text{B}_{20}$)	166	10	12
Alumina (Polycrystal)	300	19.1	4.5
Silicon (Single crystal)	168	10.0	0.7
Silicon Carbide (Polycrystal)	436	24.0	4.0
Polycarbonate	2.5	0.20	1.0
High strength aluminium alloys	80	1.8	40
Maraging steels	200	5.0	100

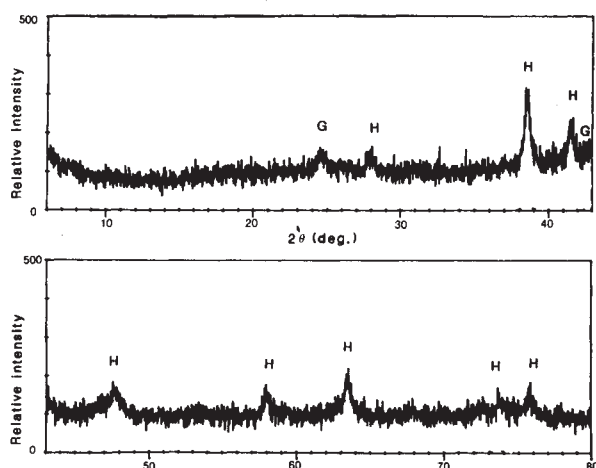


Fig. 1 A plot of X-ray diffraction analysis of the synthetic material shows that it is crystalline and consists primarily of haematite. Cobalt K- α radiation was used. H, haematite α -Fe₂O₃; G, goethite α -FeOOH.

in a controlled applied magnetic field established with three orthogonal sets of Helmholtz coils. Haematite is precipitated onto porous alumina wafers of negligible remanence with pore diameters ranging from one micrometre to one millimetre and porosity 12–14% by volume. During synthesis, the orientation of a wafer is maintained by attaching it to an epoxy base in a Nalgene bottle. Orientation arrows are drawn on the wafer and the bottle for reference; we estimate the error of orientation of the wafer relative to the magnetic field to be 10 to 15°.

The rate of haematite formation depends critically on temperature and is slow below 95 °C, but temperatures below 100 °C are necessary so that haematite crystals will not be dislodged from the wafer by boiling and to minimize thermal remanent magnetization (TRM). We heat the solution at 95 °C for six hours, remove the excess solution and dry the wafer and haematite under vacuum at 25 °C for 36 hours while maintaining their position within the applied field. The haematite-bearing wafer is removed and stored in a magnetically shielded laboratory of the Scripps Institution of Oceanography Palaeomagnetism Laboratory.

The synthesis product is dark reddish-brown and stains the wafers pervasively. X-ray diffraction analysis using cobalt K α radiation shows that it consists primarily of haematite, although some goethite is present (Fig. 1).

The Curie temperature of the material was determined by thermogravimetric analysis performed with a magnet positioned above the sample, which acts to decrease the apparent weight of the haematite as measured by the balance upon which it rests. At the Curie point, the spontaneous magnetization of the haematite is destroyed, the haematite is no longer attracted by the magnet and its measured weight increases dramatically. The Curie point thus determined for the synthetic haematite occurs at 694 °C (Fig. 2), similar to published values for haematite of 696 °C (ref. 14).

Scanning electron microscopy shows that the haematite occurs in hexagonal and anhedral crystals ranging in size from 0.1 to 1 μ m. These have nucleated on the alumina wafers and occur both as single crystals and clusters.

The results of stepwise thermal demagnetization of four wafers impregnated with synthetic haematite are shown in Fig. 3. All samples were grown in fields of 100 mT directed along the orientation arrows of the wafers and inclined upwards at an angle of 45°. Samples were grown in inclined magnetic fields to test for the acquisition of depositional remanent magnetization, which is characterized by an inclination lower than the applied magnetic field orientation in intermediately dipping fields¹⁵.

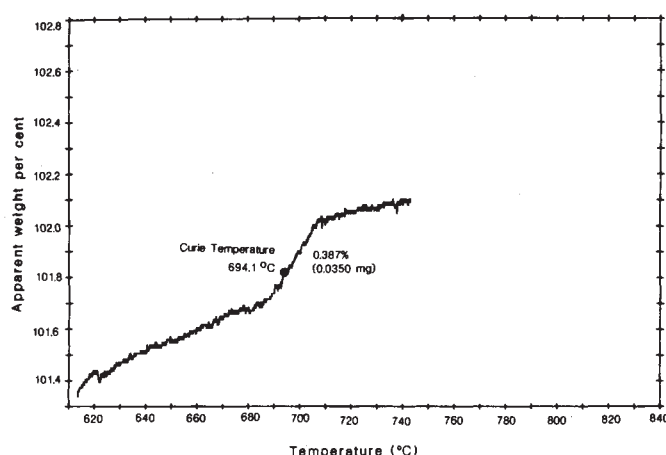


Fig. 2 Apparent weight per cent versus temperature. This analysis was performed in a strong magnetic field (10^6 A m⁻¹) at a heating rate of one degree per minute to determine the Curie temperature of the synthetic material. A value of 694 °C was obtained.

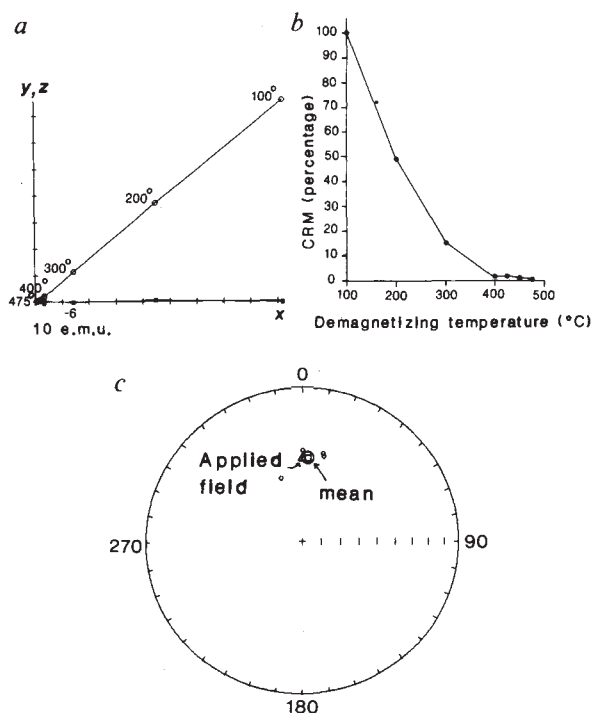


Fig. 3 *a*, Vector endpoint diagram showing the results of stepwise thermal demagnetization from 100 °C to 475 °C. Squares are plotted in the horizontal axis, circles on the vertical. The remanence acquired by the sample parallels the field applied during its growth. *b*, Per cent CRM intensity versus demagnetizing temperature shows that the blocking temperature of the material occurs between 400 and 475 °C. *c*, Equal-area projection of CRM directions (small circles), applied field direction (triangle), and the mean of the CRM directions (square). The large circle is the 95% confidence level. This shows that CRM is acquired parallel to the applied field during the formation of the haematite carrying the remanence.

Figure 3a is a vector end-point diagram showing the results of stepwise thermal demagnetization from 100 °C to 475 °C. The vector lost from natural remanent magnetization to 100 °C is a thermal remanence acquired by the sample during cooling from the synthesis temperature to room temperature and will not be considered here. Remanence revealed from 100 to 475 °C (the unblocking temperature of the material) is a grain-growth CRM, and parallels that of the applied field. The inclination recorded

by the synthetic material is approximately 40° up, similar to the applied field. Intensities are approximately 10⁻⁹ A m², several orders of magnitude higher than the noise level of our equipment (5 × 10⁻¹² A m²).

A plot of remanent intensity versus demagnetization temperature (Fig. 3b) shows that the specimen's blocking temperature occurs between 400 and 475 °C. Estimates of blocking temperature may be related to the grain size of haematite by the Neel equation^{16,17}:

$$V = (kT/K) \ln(C\tau)$$

where V is the grain volume (cubic centimetres), k is Boltzmann's constant (1.38 × 10⁻¹⁶ erg K⁻¹), T is the blocking temperature (kelvin), K is the anisotropy constant for haematite (10⁴ erg cm⁻³)¹, C is a frequency factor (10¹⁰ per second)¹, and τ is the relaxation time (10⁴ s, the time in which the sample is demagnetized).

Using these values to calculate the expected range in grain diameters of the synthetic material, where d is the diameter in centimetres:

$$V = 4/3\pi(d/2)^3 = (kT/K) \ln C\tau$$

$$d(\text{cm}) = [(\ln C\tau) (6k/\pi K) T]^{1/3}$$

For $T = 673$ K, $d = 2.67 \times 10^{-5}$ cm or 0.27 μm; for $T = 748$ K, $d = 2.76 \times 10^{-5}$ cm, or 0.28 μm. Thus, the calculated diameter of haematite whose blocking temperature is 400 to 475 °C is approximately 0.3 μm. This is at the low end of the range of grain sizes observed in the synthetic material under the scanning electron microscope. Using a value for the anisotropy constant K determined for coarse-grained haematite (3 × 10² erg cm⁻³)¹⁸⁻²⁰ calculated grain diameters of 0.8 μm are obtained, within the range of observed grain diameters.

Based on calculated blocking curves for haematite presented by Pullaiah *et al.*²¹, thermal demagnetization at 250 °C would remove any viscous remanent magnetization acquired by the sample. Therefore, the properties we attribute to CRM result from the acquisition of chemical remanence and not some other type of remanent magnetization.

In order to compare the CRM directions with the applied fields, we calculated the best-fit lines for the stepwise thermal demagnetization data by principal component analysis²². An equal area projection (Fig. 3c) of the CRM directions of these four syntheses and the applied field direction shows that the mean of the four haematite directions coincides with the applied field direction at the 95% confidence level.

The grain-growth chemical remanence carried by synthetic haematite formed in an applied magnetic field parallels the orientation of that applied field. This contrasts with previously reported properties of CRM in minerals precipitated from solution⁶ and in minerals altered from pre-existing magnetic grains^{7,9-11}.

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Identification of an underwater extraterrestrial impact crater

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When large meteorites strike the Earth's surface they form impact craters. These features have been intensively studied on land during the past two decades¹ and the geological signatures of impact events are reasonably well understood. In contrast, the effects of the impact of large meteorites into the 70% of the Earth's surface which is covered by ocean are poorly understood^{2,3}, and until now no examples have been identified. Here we report the identification of an underwater extraterrestrial impact crater on the North Atlantic continental shelf, 200 km south-east of Nova Scotia, Canada. The impact, in early Eocene time, produced a complex structure with a submarine crater, a central structural high and an inner topographic ring. The crater is filled with breccia, which exhibits shock deformation features. Lack of enrichment of the melt rocks in siderophile elements compared with basement rocks and a slight enrichment in iridium, suggest that the impactor was either a stony meteorite or a cometary nucleus. The diameter of the impactor is estimated to be ~2-3 km.

Multi-channel reflection seismic surveys off eastern Canada have demonstrated the presence of a peculiar, circular structure near the continental shelf edge south-east of Nova Scotia (42°53' N, 64°13' W). The structure was variously interpreted as of igneous origin (Union Oil Company, Montagnais I-94 Well History Report, 1975), a diatreme⁴, the result of the crossing of two major fault lineaments (A. Tankard, personal communication) and as an astrobleme (Shell Canada, personal communication). A single oil exploratory well, Montagnais I-54, located over the centre of the structure in 1974 was reported to have encountered a thick conglomerate and breccia beds with several horizons of basaltic rocks (Union Oil Company, Montagnais I-94 Well History Report, 1975).

The structure is located near the shelf edge in a water depth of 113 m. The seismic profiles show a crater at least 45 km in diameter, with a central irregular uplift 1.8 km high and 11.5 km in diameter. The projected depth of the centre of the crater is about 2.8 km, shallowing to the edges. The crater is partially infilled by a seismically isotropic mass, interpreted to be formed by fallback breccia (Fig. 1). The breccia layer, which in the crater is thinner over the central uplift, is up to 850 m thick and surrounds the central structural uplift up to a distance of 8.5 km. The top of the fallback breccia is overlain by Tertiary marine deposits that buried the crater. The crater was subsequently eroded when currents, probably deflected around the central high, incised deep channels into the post-crater fill (Fig. 1).

Three major units were penetrated in the Montagnais I-94 well (Fig. 2). At 653 m below the sea floor a sequence of marine Cenozoic sediments overlies the second unit with sharp contact, represented by 552 m of polymictic breccia that includes two horizons of partial and recrystallized melts (previously interpreted as basalts) and an upper suevite zone. The breccia in

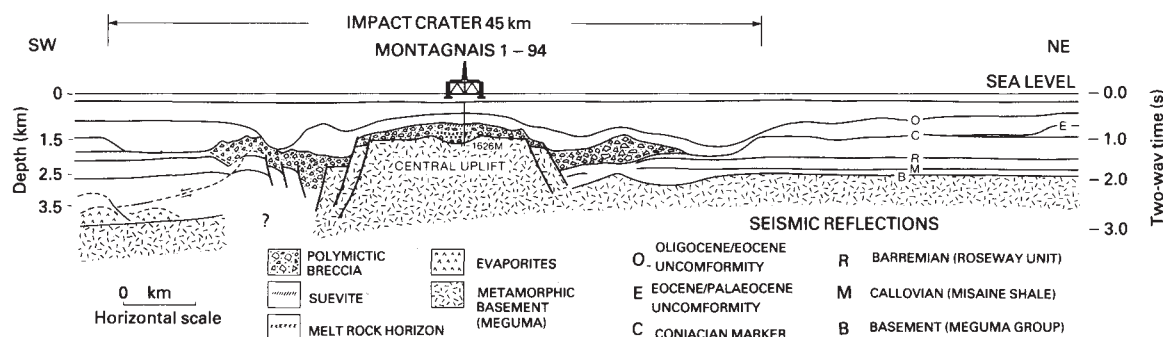


Fig. 1 Interpreted multi-channel reflection seismic section of the Montagnais impact structure. The seismic line connects two oil exploratory wells on the Scotian Shelf, the Mohawk B-93 and Montagnais I-94. The seismic line 3203-82 has been provided by Petro-Canada and partners.

turn overlies the third rock unit which is a low-grade metamorphic basement complex consisting of chloritic subgraywacke and phyllite with beds of metaquartzite, dipping 50° SE, similar to the Meguma Group of Nova Scotia⁵. Thin sections of the basement rocks sampled by conventional core show hair-thin fractures and presence of planar lamellae in some quartz and feldspar grains, particularly near the top of the basement sequence. Locally, birefringence in quartz is reduced. These features are characteristic of mild shock metamorphism and shock pressure of 10 GPa⁶.

The polymictic breccia unit has been examined from well-cutting samples. It consists principally of Meguma basement lithologies. Near the base of the breccia zone, which is interpreted to represent the bottom of the excavated cavity, are traces of quartz sand and light grey mudstone, mixed with Meguma clasts. In the upper 160 m of breccia unit, the basement clasts are highly epidotized and mixed with quartz sand, mudstone and clasts of limestone and granite. The limestone is similar to Jurassic limestone and the granite is similar to basement at 2,112-m sub-bottom in the nearby Mohawk B-93 well. The size of clasts in the breccia cannot be determined from cutting samples, although locally monomictic composition of the cuttings suggests the presence of blocks several tens of metres in size. The breccia contains clasts with occasional shock lamellae in quartz (Fig. 3a) and feldspars, local development of maskelynite in feldspars and lechatelierite in quartz, melted rock with frothy fabric and glassy streaks and schlieren.

The melt zones (71 m and 35 m thick) are similar in appearance, with the lower part of the melt zone represented by dark grey to blackish fine crystalline rock consisting of plagioclase (of mostly labradorite-bytownite composition), rare sanidine, quartz, magnetite and apparently devitrified glass matrix (Fig. 3b). The upper part of both melt zones is a mixture of variably melted clasts of graywacke, which show partial melting and the presence of diaplectic glass, clasts with outer glass envelopes, originally glassy feldspars recrystallized into spherical aggregates, and clasts with frothy fabric. The proportion of melted clasts in the upper part of the melt zone decreases stratigraphically upwards; conversely clasts of polymictic breccia with shock metamorphic features such as intensive planar lamellae and low birefringence patches, become more common.

The top of polymictic breccia unit consists of a 40-m thick suevite zone. It is composed of highly vesicular glassy particles (Fig. 3c), many with flow texture and the fusion of several clasts (Fig. 3d). Unabsorbed clastic grains enclosed in glass are common. One-third of the clasts are recrystallized into microcrystalline aggregates.

Chemically, the melt rocks are similar to rhyolites in silica content (Table 1), but not in other elements. Also, the plagioclase are bytownite - labradorite, not the oligoclase normally found in rhyolites. The major and trace element composition of the melts is remarkably similar to that of analysed Meguma basement rocks from the well (Table 1), although they are enriched

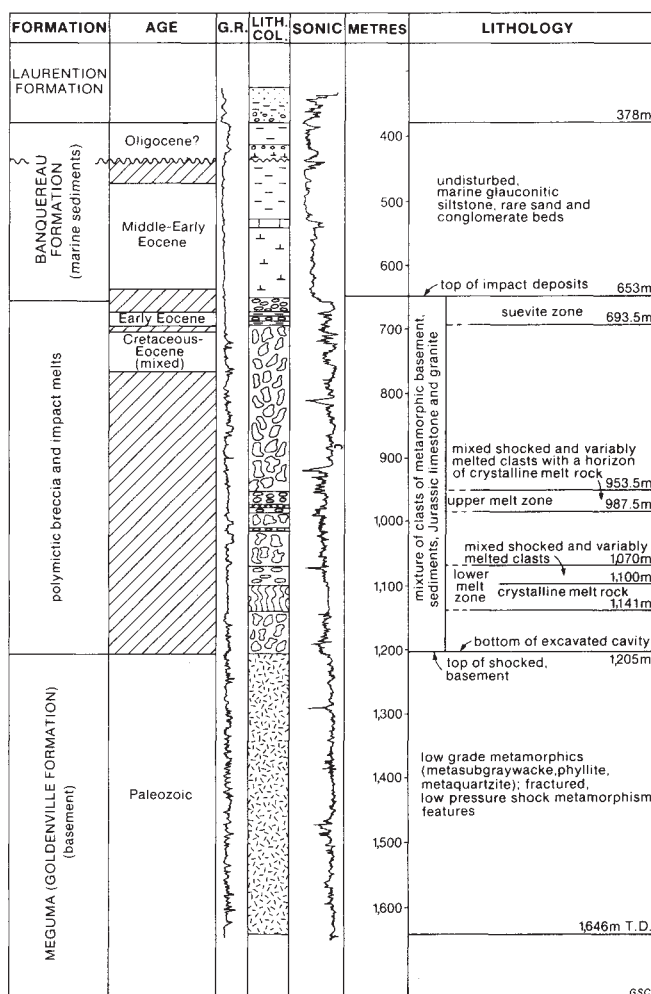


Fig. 2 Vertical stratigraphic column of the Union Oil Company Montagnais I-94 oil exploratory well. The well is located at the centre of the impact structure (see Fig. 1) and encountered three main lithological units, slightly shocked Palaeozoic basement, zone of shocked breccia and impact melts and undisturbed cover of Cenozoic marine sediments. G.R., gamma-ray log; Lith. col., generalized lithological column.

in silica and potassium and depleted in aluminium, sodium and magnesium; a tendency noted in other impact melts⁷. Of the siderophile elements, iridium concentration (0.22 p.p.b.) is an order of magnitude higher than in the basement rocks (0.016 p.p.b.) and as much as two orders of magnitude higher than expected for continental shelf sedimentary rocks. But it is several orders of magnitude lower than concentrations of iridium in carbonaceous chondrites and iron meteorites. Similar iridium

Table 1 Chemical analyses of melts and metamorphic basement in Montagnais I-94 well

	Melts		Metamorphic basement	
	Upper 978-987 m	Lower 1,109-1,118 m	1,600-1,609 m	1,627-1,636 m
SiO ₂	74.66	75.13	71.08	70.58
TiO ₂	0.50	0.72	0.59	0.68
Al ₂ O ₃	12.82	12.54	15.20	15.10
FeO _t	3.70	3.45	4.99	5.22
MnO	—	—	0.06	0.01
MgO	1.32	1.26	1.62	1.87
CaO	1.63	2.06	0.78	1.04
Na ₂ O	2.32	2.11	2.60	2.56
K ₂ O	2.97	2.75	2.41	2.86
P ₂ O ₅	0.03	0.03	0.04	—
Total	99.95 (30)	100.05 (20)	99.37 (10)	99.92 (10)
Ni*	31	32	29	35

* Analyses by atomic absorption method, values in p.p.m.
Number in parentheses, number of analyses.

concentrations in other impact crater deposits have been explained as resulting from cometary impacts⁸.

Whole rock K-Ar isotope dating of the basal melt horizon yielded ages of 49.9 ± 2.1 and 55.8 ± 0.9 Myr from two different laboratories, bracketing the impact to the late Palaeocene - early Eocene. Dinoflagellates in the overlying siliceous, glauconitic mudstone indicate an early to middle Eocene age (E. Davies, Geological Survey of Canada, Internal Report, 1984). There is no evidence from overlying sediments that the top of the structure was at any time subaerially exposed, even though slightly coarser sediment over the top of the structure suggests that it was shallower than the coeval horizon off the structure.

It has been suggested that an impact event in the ocean will produce different, but unspecified structures because of the coupling of the shock wave with water¹, or that because water has zero strength it cannot preserve a crater form⁹. The Montagnais structure shows that the crater form resulting from an impact of a bolide into a shallow sea is well preserved. The Montagnais structure is not a simple crater; it is a complex crater similar to those recognized on land, in that the central core is uplifted and shocked rocks are surrounded by one or more concentric depressions.

Roddy¹⁰ and Pike¹¹ suggested that the formation of complex craters is related to the shallow penetration from the impact of a low-velocity cometary body, a hypothesis disputed by Grieve *et al.*¹². Geochemical data give support to the Montagnais complex crater having been formed by the impact of either a stony meteorite of Apollo type or by a cometary nucleus even though much more extensive geochemical research is needed to establish the composition of the impacted body with certainty. The size of the crater suggests that the impactor was a body of about 2 to 3 km in size¹³. The impact resulted in pressures of 50-60 GPa and post-shock temperatures of 1,300-1,600 °C at the base of the excavated cavity⁶. The complex erosion around the crater periphery might be the result of the back flow of sea water as tsunami-like waves were generated as a consequence of water-column disturbance by shock waves. The crater area was modified later by sea-bottom processes affected by the presence of the basement high and differential subsidence of the sedimentary fill of the crater.

The almost continuous sedimentary record in the surrounding shelf area, if properly sampled, will provide a unique opportunity to investigate the effect of major extraterrestrial body impact on the marine ecosystems and the sedimentary record and to provide constraints on the hypothesis of biological mass extinction as a result of meteoritic impacts.

We thank Petro Canada and A. Tankard for providing multi-channel seismic data, D. Sherwin and COGLA for permission

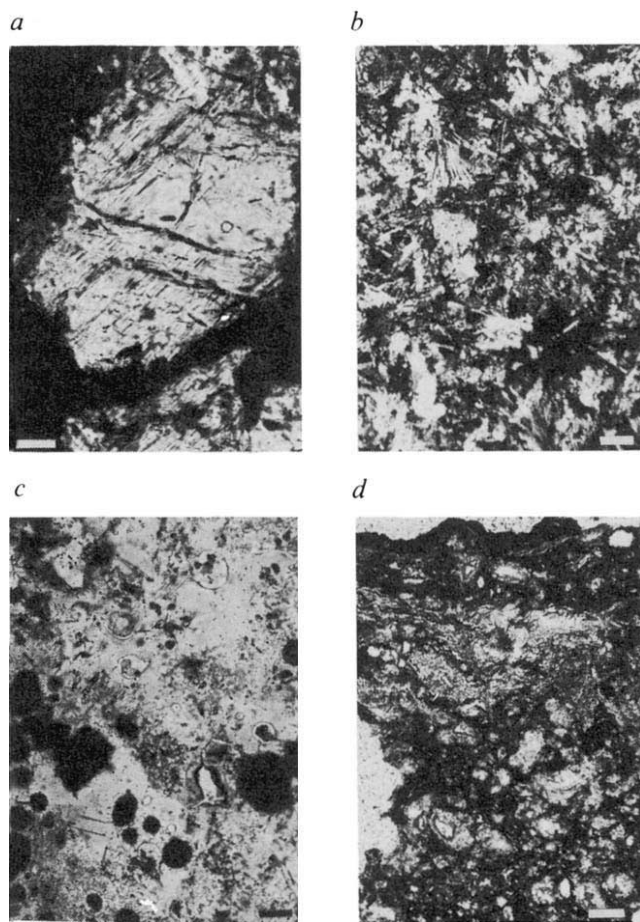


Fig. 3 *a*, Highly shocked quartz grain from the polymictic breccia. The grain exhibits several non-decorated planar lamellae sets and microfractures. Montagnais I-94, 698 m. Scale bar, 0.2 mm; crossed polarizers. *b*, Crystallized melt from the lower melt zone in the breccia composed of radial aggregates of feldspar microliths, minute opaque minerals (magnetite), rare chloritized biotite crystals in dusty devitrified glass matrix. Montagnais I-94, 1,127 m. Scale bar, 0.2 mm; crossed polarizers. *c*, Melt glass in the breccia from the suevite zone. Thin section shows inhomogeneous texture of the glass with globular opaque inclusions and rounded vesicles. Montagnais I-94, 661 m. Scale bar, 0.02 mm; plane polarized light. *d*, Suevite, heterogeneous aggregate of partially melted particles with an outer glassy envelope with flow texture, suggesting that the aggregate is a bomb. Montagnais I-94, 698 m. Scale bar, 0.1 mm; polarized light.

to sample drill cuttings. We thank C. Orth for Ir analysis and J. Wade, B. MacLean and F. Robertson for useful discussions. D. Piper, J. Dostal and H. Palme provided valuable comments, improving the manuscript. This study was supported by EMR and NSERC and by the Geological Survey of Canada.

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Rapid shifts in phosphate acquisition show direct competition between neighbouring plants

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Direct demonstrations of competition between higher plants for specific soil resources are seldom possible¹⁻³. Previous neighbour plant removal experiments in desert environments have shown increased growth and improved water status of the remaining plants⁴⁻⁶. However, these responses were only measured several months after the removal of neighbours, after which period there would have been ample time for major adjustments in the root distribution of surviving plants. We report here field experiments using dual-isotope labelling to measure opportunistic phosphate acquisition by shrubs within 2 weeks of partial defoliation of neighbouring grass plants. Phosphate isotope uptake from interspaces shared with defoliated grasses increased to as much as six times that of uptake from interspaces shared with unperturbed neighbours. The experiments indicate immediate competition for phosphate and the influence of root physiological activity on this competition.

We conducted two field experiments with *Artemisia tridentata* ssp. *vaseyana* (Rydb.) Beetle, a dominant shrub in western North America. One experiment was with a co-occurring grass, *Agropyron spicatum* (Pursh) Scribn. and Smith, and one with *Agropyron desertorum* (Fisch. ex Link) Schult., a grass from Eurasia widely seeded in this region. These two tussock grasses have very similar canopy structure, phenology, photosynthetic characteristics and biomass allocation⁷⁻¹¹. Yet, *A. desertorum* is more competitive and more tolerant of grazing^{3,7,12,13}. All three species have vesicular-arbuscular mycorrhizae of the genus *Glomus*³.

To provide replicate plant sets, the experiments were conducted in field plots established with transplants seven years earlier. Soils are Typic Haploxerolls⁷ and were not ploughed before transplanting. Bicarbonate-exchangeable phosphate is generally <6 p.p.m. The shrubs were interplanted with either grass species in an even 0.5-m spacing. Their roots intermingle extensively^{3,7,13}. The experiments with the two grass species were in adjacent plots. Six replicate plant sets, each consisting of a shrub surrounded by four grass plants, were chosen for

Table 1 Total quantities of P isotope in neighbouring grasses

<i>Agropyron spicatum</i>			
	Control (kBq)		Defoliated (kBq)
Shoots	380 (92% ± 2%)	Regrowth shoots	12 (78% ± 5%)
		Older shoots	3 (19% ± 5%)
Crowns	35 (8% ± 2%)	Crowns	0.4 (3% ± 0.6%)
Total	415 ± 107	Total	15.4 ± 6
<i>Agropyron desertorum</i>			
	Control (kBq)		Defoliated (kBq)
Shoots	686 (94% ± 2%)	Regrowth shoots	250 (86% ± 2%)
		Older shoots	36 (12% ± 2%)
Crowns	47 (6% ± 2%)	Crowns	7 (2% ± 0.4%)
Total	733 ± 234	Total	293 ± 115

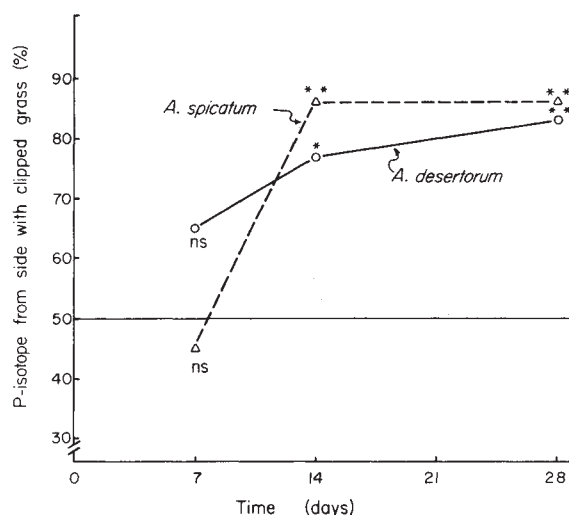
Total quantities of P isotope in kBq (± one s.e.m.) and percentage allocated to plant parts (± one s.e.m.). In the experiment with each grass species $n = 6$.

each experiment. The grass tussocks had basal diameter ~15 cm and the shrubs had canopies ~30–50 cm in diameter. Total canopy cover was similar to that in shrub/grass steppe communities. All three species were actively growing at the time of the experiments. Soils were moist throughout the rooting zone which is common at the time of year of the experiments^{7,12}. On opposite sides of each shrub, ³³P and ³²P isotopes (18.5 MBq each, as carrier-free orthophosphoric acid) were injected into a series of ten 30-cm-deep holes previously created by inserting 6-mm-diameter rods into the soil. The holes were 2.5 cm apart in a row midway between the shrub and the grass and perpendicular to a line connecting the plants. The holes were then covered by soil. Phosphorus desorption tests indicate that 90–95% of added P is bound in these soils. The amount of total P injected in each row was less than 10⁻¹¹ g cm⁻³, which is below the concentration that might increase root growth¹⁴.

On the day before labelling (23 May 1985), one of the grasses adjacent to a labelled interspace was clipped to 7 cm in height (~85% foliage removal). The clipped plant was next to a ³²P label in half the replicates and next to a ³³P label in the other half to compensate for possible isotope discrimination.

After 53 days, the experiments were terminated and the grasses harvested to estimate the P-isotope content of the shoots and crowns. Subsamples reduced to ashes (500°C) were digested in HCl, and scintillation counts were corrected for half-life, counting efficiency and energy overlap. As expected, the clipped

Fig. 1 The proportion of P isotope acquired by *Artemisia* from the labelled interspace shared with the partially defoliated grass at different times following defoliation and labelling. Each point represents the isotope proportion in the preceding time interval. This is shown for separate, but concurrent, experiments with both tussock grass species. As the two experiments were conducted in adjacent plots, comparison of the grass species is not appropriate¹⁹. Results of *t*-tests with square-root arcsin-transformed data at each time in each experiment are indicated thus: **, $P < 0.01$; *, $P < 0.05$; ns, not significantly different from the null hypothesis of 50% isotope from clipped side; $n = 6$ for each experiment.



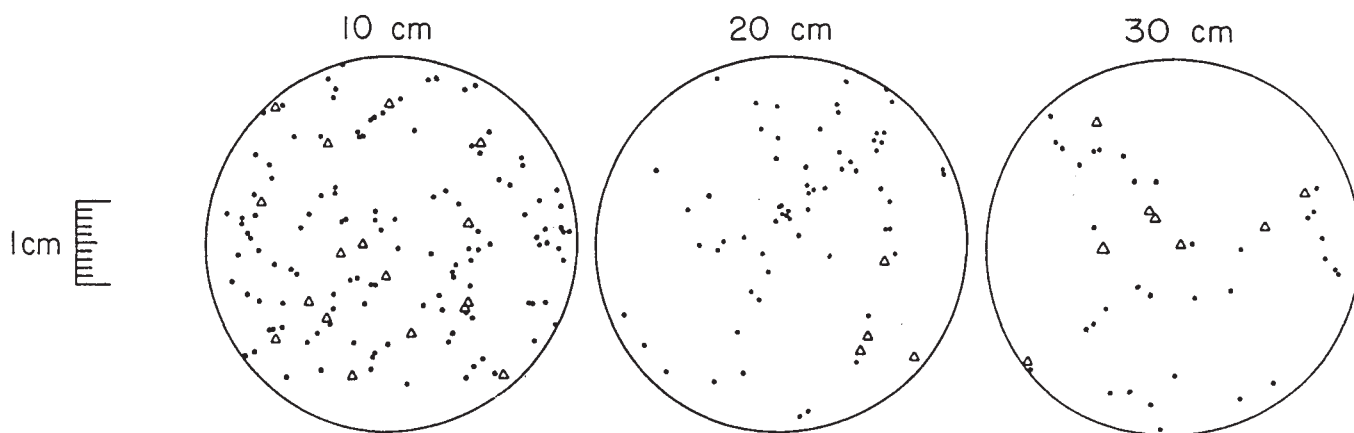


Fig. 2 The distribution of individual fine roots in cross-sections of soil in the interspace between *Artemisia* (Δ) and *A. desertorum* ($\bullet$) at three depths. Parcels of soil previously frozen with liquid nitrogen were excavated and then sectioned at -70° with a lapidary saw. The position of individual roots intersecting the cut plane was mapped from the cut soil sections and from enlarged photographs of them. To determine whether the individual roots belonged to *Artemisia* or the grass, a fluorescence technique has been developed and tested on roots of *Artemisia* and *Agropyron* of different age and phenological status. On partial thawing of the soil, individual root segments (~ 1 cm) were removed from the soil and dried. A basic extract of the roots (2N NaOH) was dried on chromatography paper and the spots observed under a UV-A lamp. The *Artemisia* and *Agropyron* roots are clearly distinguished by colour and intensity of fluorescence.

grasses acquired less isotope than the unperturbed grasses even though they were rapidly regrowing (Table 1).

If the shrubs and grasses are competing for the same resource at the same time, reduced uptake by the grass should be reflected in increased resource acquisition by the shrub. The relative P-isotope acquisition by the shrub from the two labelled interspaces was determined by the ratio of P isotopes in small shoot samples at different times^{3,15}. Within two weeks following defoliation, the proportion of P isotope acquired by *Artemisia* from the interspaces shared with the clipped grasses increased substantially (Fig. 1). This response was very similar in experiments with both grass species, even though these grass species differ greatly in their ability to recover from defoliation and to compete^{3,7,12,13}. After 53 days when the grasses were harvested, the proportion of P isotope acquired by the shrubs from the interspaces shared with the clipped grasses was 68 and 76% in the experiments with *A. spicatum* and *A. desertorum*, respectively (both significantly different from 50% at $P < 0.05$; Wilcoxon rank sum test).

Competition for P is very localized. Because phosphate ions are almost immobile in soils, the effective uptake zone is within a few millimetres of a root and its associated mycorrhizae¹⁶. Thus, individual roots of neighbouring plants must be very close to each other to compete for the same phosphate ions at the same time. The distance between individual roots depends both on the average rooting densities (root length divided by soil volume) and the distribution patterns of individual roots. As shown in Fig. 2, individual roots of neighbouring plants were close enough to compete directly for phosphate.

The proximity of the neighbouring plant roots and the rapid and sizeable shift in P uptake exhibited by the shrub indicate an immediate competition for P. This competition, in turn, appears to be substantially influenced by the physiological activity of the roots rather than simply by general root morphology and distribution in the soil. Processes such as root phosphate absorption, continued fine-root maintenance and extension, and support of mycorrhizae all depend on a continuing energy supply to the roots and on plant demand for P. Treatments such as shading and defoliation can curtail root

growth and nutrient uptake within 24 h (refs 17 and 18). In our experiments, the reduced P absorption by the clipped grass plants (Table 1) was rapidly reflected in the acquisition patterns of the shrub (Fig. 1) even though the physiological activity and general distribution of the shrub roots would not be directly affected by the clipping treatments.

The rapid shift in resource acquisition demonstrated in these experiments not only indicates immediate resource competition, but also shows how quickly the balance of competition might change in the event of herbivory. We have focused on competition for phosphorus because of the sensitivity of the dual-isotope approach. We do not, however, feel that P is the only, nor necessarily the pivotal, resource in the competitive balance of these plants³.

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Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels

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Recent measurements of atmospheric CO₂ levels in ice cores¹ have shown that global CO₂ has increased by about 60 $\mu\text{mol mol}^{-1}$ over the past 200 years. Evidence for the response of plants in the field to this change in CO₂ levels is here presented in the form of a significant change in stomatal density—an anatomical response of considerable ecophysiological importance. A 40% decrease in density of stomata was observed in herbarium specimens of leaves of eight temperate arboreal species collected over the last 200 years. This decline was confirmed for some of the species observed as herbarium specimens by experiments under controlled environmental conditions. In these an increase in the mole fraction of CO₂ from 280 $\mu\text{mol mol}^{-1}$ to the current ambient level of 340 $\mu\text{mol mol}^{-1}$ was found to cause a decrease in stomatal density of 67%. Experiments have shown that the combination of this previously unreported response of stomatal density to the level of CO₂, with the known responses of stomatal aperture², cause water use efficiency to be much lower than expected at low levels of CO₂ and over a wide range of humidities.

The opening of stomata, usually measured as a conductance to water vapour of a unit area of leaf, is very sensitive to a range of environmental conditions². In some cases, such as the responses to irradiance and drought³, these short-term responses of stomatal aperture occur in addition to variations in the numbers of stomata per unit area, the stomatal density, a feature which is established during an early stage of leaf development.

It has recently been found that the increases in stomatal

Table 1 Response of stomatal index to changes in CO₂ mole fraction

Species	CO ₂ mole fraction ($\mu\text{mol mol}^{-1}$)	Stomatal index (%)	
		Adaxial surface	Abaxial surface
<i>Acer pseudoplatanus</i>	225	0	14.9 $\pm$ 0.8
	340	0	6.7 $\pm$ 1.1
<i>Quercus robur</i>	225	0	17.4 $\pm$ 1.1
	340	0	9.6 $\pm$ 1.0
<i>Rumex crispus</i>	225	3.5 $\pm$ 0.8	15.5 $\pm$ 0.7
	340	0.5 $\pm$ 0.3	11.8 $\pm$ 0.9
<i>Vaccinium myrtillus</i> ⁵	250	13.1 $\pm$ 1.9	17.5 $\pm$ 2.4
	340	2.5 $\pm$ 0.8	16.9 $\pm$ 2.2
<i>Lycopersicon esculentum</i> ⁹	450	1.2 $\pm$ 1.6	14.2 $\pm$ 2.7
	350	26.4	31.0
<i>Liquidambar styraciflua</i> ¹⁰	650	25.6	29.5
	1,000	26.6	27.6
	340	0	25 $\pm$ 6.8
	520	0	28 $\pm$ 4.5
	718	0	29 $\pm$ 6.8
	910	0	26 $\pm$ 4.5

Values are means $\pm$ s.e.m. Stomatal index = (stomatal density)/(stomatal density + epidermal cell density) $\times$ 100.

density with altitude, which have been observed for some species on European mountains⁴, can be simulated by growing plants in the reduced partial pressures of CO₂ expected at altitude⁵. The possibility that changes in the levels of atmospheric CO₂ over the past two centuries¹ may also have affected the stomatal densities of a wide range of plants at low altitudes was therefore investigated.

Stomatal densities were measured on the leaves of seven species of temperate forest trees and one species of shrub, which had been collected over the last 200 years and stored as dried herbarium specimens. Although the absolute stomatal densities

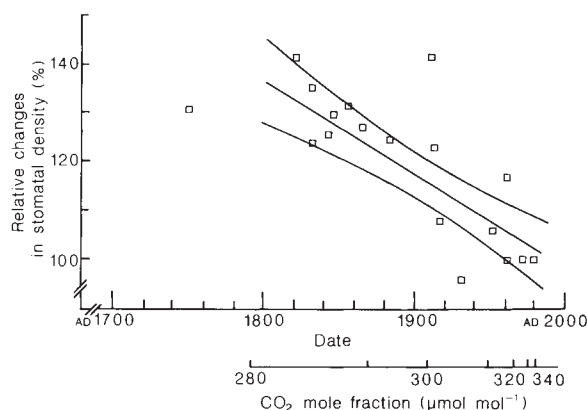


Fig. 1 Abaxial stomatal densities of herbarium stored leaves of *Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Populus nigra*, *Quercus petraea*, *Q. robur*, *Rhamnus catharticus* and *Tilia cordata*. Leaves had been stored in the herbarium in the Department of Botany, University of Cambridge. Only leaves on reproductive shoots were sampled, with the assumption that these leaves had developed in full irradiance. Five leaves of each species were sampled from different dates, back to AD 1750, and from collections made in the midlands of England. Stomatal densities varied between species by a factor of about two, however the changes in stomatal densities relative to the recent collections (1970 to 1981) were similar for all species. Reconstructed changes in atmospheric CO₂ based on ice-core studies¹ are also included. The linear regression line, with 95% confidence limits, shows a 40% reduction in the ratio of stomatal densities over a period of 200 years, $r = -0.828$.

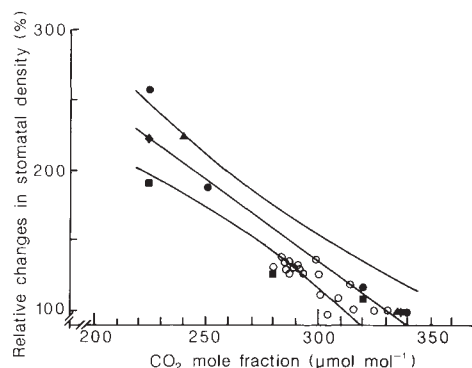


Fig. 2 Comparison of the experimental effects of a change in the CO₂ mole fraction on stomatal density with the putative effects of CO₂ shown in herbarium material (○). Experimental observations were made on *Acer pseudoplatanus* (●), *Quercus robur* (◆), *Rhamnus catharticus* (▲) and *Rumex crispus* (■). Plants were grown in small air tight enclosures for a period of three weeks in a 16-h photoperiod at 18 °C, a mean leaf water vapour pressure deficit of 1.2 kPa and an irradiance of 373 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The night temperature was 7 °C. The CO₂ mole fractions supplied to the enclosures was controlled with a gas diluter (Analytical Developments) and measured with an infra-red gas analyser (ADC). The linear regression for the experimental observations, on leaves which had been initiated and developed in the treatments, had a slope of $-1.12 \pm 1.5\% \mu\text{mol}^{-1} \text{mol CO}_2$ (95% confidence limit) ($r = -0.940$). The linear regression coefficient for the herbarium material (○) was $-0.61\% \pm 0.8\% \mu\text{mol}^{-1} \text{mol}$ ($r = -0.858$).

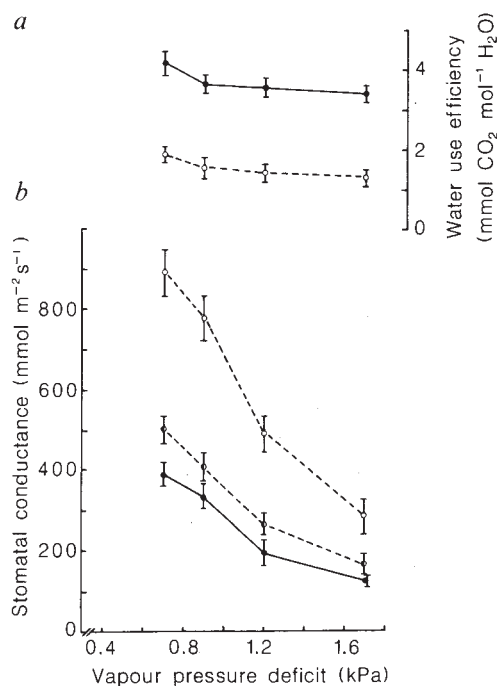


Fig. 3 The responses of *a*, WUE (the ratio of instantaneous rates of photosynthesis to transpiration) and *b*, stomatal conductance in *Acer pseudoplatanus* to variations in the water vapour pressure deficit of the air. ○, Measurements on leaves grown at a CO₂ mole fraction of 225 μmol mol⁻¹; ●, measurements on leaves grown at a CO₂ mole fraction of 340 μmol mol⁻¹; ◐, measurements on leaves grown at a CO₂ mole fraction of 340 μmol mol⁻¹ and then transferred to a mole fraction of 225 μmol mol⁻¹ for one week before measurements (the stomatal density of measured leaves was not significantly different from leaves maintained continually at 340 μmol mol⁻¹). Measurements of transpiration, stomatal conductance and photosynthetic rate were made with a portable gas analyser (Type LCA, Analytical Developments) at different water vapour pressures in the environments for growth (Fig. 2).

differed between species, a similar relative decrease in density was found in all cases (Fig. 1) with a mean reduction of 40% between AD 1787 and the present. Over the same period the ice record suggests that the CO₂ in the atmosphere has increased by 60 μmol mol⁻¹ (ref. 1) or 21%.

Three of the species studied as herbarium material and two additional, herbaceous species were grown in controlled environment conditions at a range of CO₂ mole fractions between 225 μmol mol⁻¹ and the current level of 340 μmol mol⁻¹, to investigate the responses of stomatal density to CO₂.

The experimental evidence (Fig. 2) demonstrates that variations in the mole fraction of CO₂ alone can regulate stomatal density with a mean decrease of 67% resulting from the 60 μmol mol⁻¹ increase in CO₂.

In many cases where drought and irradiance influence stomatal density, they do so by affecting the expansion of leaf area, causing the stomata to be packed more densely (drought) or less densely (low irradiance) through changes in the size of the epidermal cells surrounding the stomata³. Thus when the effects of epidermal cell size are taken into account in the stomatal index (defined in Table 1) the treatment effects may largely disappear, indicating that the response simply involves the control of epidermal cell expansion^{3,6}. In the experiments described here however, the changes in stomatal density were very similar to changes in stomatal index (Table 1), indicating that the effect of CO₂ is on stomatal development. In the case of the herbarium specimens it was not possible to measure stomatal index reliably. Note that neither stomatal density nor stomatal index responded to CO₂ levels exceeding the current level.

The response of stomatal density reported here indicates an apparently unknown effect of CO₂ on plants. Its significance centres on the control of leaf gas exchange (Fig. 3). The high stomatal density which develops at low CO₂ increases stomatal conductances at low water vapour pressure deficits. For example, as shown experimentally (Fig. 3), the stomatal conductance of *Acer pseudoplatanus*, at a vapour pressure deficit of 0.7 kPa and a CO₂ mole fraction of 225 μmol mol⁻¹ is 2.3 times greater than at 340 μmol mol⁻¹. The effect of CO₂ on stomatal aperture is well established² but the effect of this response alone on stomatal conductance is much less, with a 1.3-fold increase in conductance as the mole fraction of CO₂ is decreased from 340 to 225 μmol mol⁻¹. Although stomatal conductance decreases as the water vapour pressure deficit increases, the relative change in conductance between the CO₂ treatments was found to remain more or less constant.

The differential effect of the response of stomatal density to CO₂ on both photosynthesis and transpiration is shown by calculations of the water use efficiency (WUE, Fig. 3). At a CO₂ mole fraction of 340 μmol mol⁻¹ and a vapour pressure deficit of 0.7 kPa, WUE is 2.2 times greater than at 225 μmol mol⁻¹; the difference was insensitive to changes in the vapour pressure deficit.

These measurements are as expected from the linear response of stomatal density to CO₂ (Fig. 2) and suggest that in the lower CO₂ levels of the pre-industrial era the uptake of CO₂ should have been more expensive in terms of water loss than the present day. According to the optimization theory of stomatal control⁷ this would have caused reductions in stomatal conductance and leaf area expansion, to reduce water loss and the incidence of drought, and maintain dry-matter production⁸.

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Relation of neurons in the nonprimary motor cortex to bilateral hand movement

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In the primate cerebral cortex there are at least two somatotopically organized, nonprimary motor fields rostral to the primary motor area^{1,2}. To understand the functions of these multiple motor representations we have compared the neuronal activity in each of these fields while monkeys performed a trained motor task, using right, left or both hands. In the nonprimary motor cortex, activity in a number of neurons was related to the movement the animal chose and performed, whereas in the primary motor cortex, changes

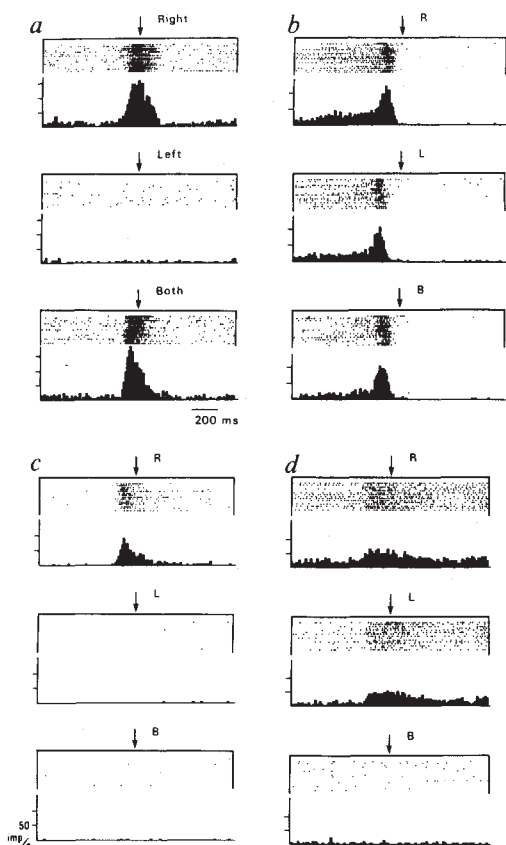


Fig. 1 Discharge of a primary motor cortex neuron (*a*), and three nonprimary motor cortex neurons (*b, c, d*) exhibiting different relationships to key press movements performed with right hand (top row), left hand (middle) and with both hands (bottom). Neuronal activity is displayed in two different formats. Top, rasters with each row representing a trial and dots representing individual single-cell discharges. Arrows correspond to movement onset. Bottom, histograms representing summation of data in rasters.

in the firing of most neurons were simply related to activity in the contralateral muscles. This result indicates that the nonprimary motor cortex is involved in higher-order coding of the laterality of the motor response, implying that it exerts its motor control function at a higher hierarchical level³ than its counterpart in the primary motor cortex.

Anatomical and physiological studies have indicated that the relation of the primary motor cortex of primates to distal limb movements is largely or almost exclusively contralateral^{4,5}. What, then, is the relation of the nonprimary motor cortex? Prompted by histological studies revealing more bilateralized afferent and efferent fibre connections of the nonprimary areas^{6,7}, we initially attempted to compare the neuronal activity related to ipsilateral and contralateral digit movements. In the process of this study, some properties of neuronal activity fundamentally different from those hitherto described in cortical motor areas were found. In a number of nonprimary motor cortex neurons, the activity is related to whether the movement is to be ipsilateral, contralateral or bilateral, rather than whether the movement involves muscles in the ipsilateral or contralateral hand.

Two Japanese monkeys were trained to press a small key with the right or left hand, or with both hands, in accordance with visual instruction signals given on the front panel. A signal (given by an LED) on the right or on the left meant that the subsequent movement was to be the right or left key press. Both signals simultaneously meant that the keys had to be pressed simultaneously with both hands. The instructions were given 2.6–5.4 s before a go signal, an LED placed in the centre of the

panel. As the training progressed, the key was reduced gradually in size, until it was to be pressed with 3 (2nd to 4th) digits. Great care was taken to train the animals to use only the required part of the limb, properly adjusting the mode of fixation of the palm, wrist, elbow and shoulder. After extensive training, electromyographic studies revealed that muscle activities before the key press were limited to the digit and hand muscles of the limb instructed to move. No overt muscle activities detectable in proximal forelimb, shoulder, chest, back or hindlimb muscles were time-locked to any of the key press movements. This was further confirmed by the absence of perimovement activity changes in the precentral and postcentral cortical neurons recorded from body and proximal limb areas. After finishing the training, neuronal activities were recorded from the hand areas of the primary motor cortex, premotor cortex (PM)⁸ and supplementary motor area (SMA)⁹.

The great majority (85%) of the 241 neurons recorded from the digit area of the primary motor cortex (mostly limited to the anterior bank of the central sulcus) showed a contralateral relationship, namely the activity increased or decreased before the onset of the contralateral and bilateral key-press movement. For most, the magnitudes of the changes in activity preceding contralateral and bilateral movement were not different (see Fig. 1*a* for example). Eight per cent of the primary motor cortex neurons showed an ipsilateral relation (activity changes before ipsilateral and bilateral key press). Thus, although there are exceptions, the activity of the great majority of the primary motor cortex neurons appeared to be akin to that of hand muscles.

The most striking finding in the non-primary motor cortex was the presence of a large number of neurons (in both SMA and PM) whose relationship to the motor task were entirely different from that observed in any muscles. Of particular interest was the exclusive relationship observed in 32 SMA and 18 PM neurons (out of 119 SMA and 101 PM movement-related neurons). Some of the neurons (16% of the exclusive neurons) were active before the key press movement with the right hand but not with both hands (an example with a SMA neuron is given in Fig. 1*c*). Others (20%) were active before the left key press but not the bilateral key press. Still others (44%) were active before the bilateral key press but not before the right or left key press. There were also cells (20%) whose activity changed before either the right or left key press but not before the bilateral key press (Fig. 1*d*). In addition to these exclusively related neurons, a separate category of neurons (38% of SMA and 48% of PM movement-related neurons) exhibited activity changes before all types of movements. An example of a PM neuron with such nonselective relationship is displayed in Fig. 1*b*. Among the nonprimary motor cortex neurons the contralateral relationships, most frequently observed in the primary motor cortex, were in the minority. In addition to the period immediately preceding movement onset, activity changes were also observed in the period following the occurrence of the instruction signals, long before the movement onset. In the primary motor cortex, the instruction-induced activity changes were mostly related to the contralateral key press. This was not the case in the nonprimary motor cortex. In both SMA and PM, the instruction-induced activity changes were often related exclusively or preferentially to one of the key press movements.

These findings indicate that the majority of nonprimary motor cortex neurons do not code the activity of a particular muscle or muscles. A considerable number of these neurons seem to be related to the movement the animal is performing. This deviation of neuronal activity from that of skeletal muscle, and the closer relationship of such neuronal activity to the movement itself (in this particular motor task the usage of the right or left hand, or both hands) are characteristic properties of the nonprimary motor fields. The activity property seems to indicate a manner in which the nonprimary motor area represents more abstract nature of the movement than the primary motor cortex.

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Noradrenaline and β -adrenoceptor agonists increase activity of voltage-dependent calcium channels in hippocampal neurons

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The predominance of unconventional transmitter release sites at noradrenaline-containing synapses and the diffuse projections of noradrenaline-containing fibres originating in locus coeruleus¹ have led to speculation that noradrenaline may act as a neuromodulator^{2–6} in the central nervous system. Evidence suggests that it has a modulatory function in the plasticity of the developing nervous system^{7,8}, in controlling behavioural states of an organism^{3–6}, and in learning and memory^{9–11}. Recently, Hopkins and Johnston¹² demonstrated that noradrenaline enhances the magnitude, duration and probability of induction of long-term potentiation (LTP) at mossy fibre synapses in the hippocampal formation, and LTP is widely believed to be a cellular substrate for aspects of memory^{13,14}. To investigate the membrane effects of noradrenaline on central neurons, we used a newly developed preparation in which patch-clamp techniques can be applied to exposed adult cortical neurons. We report here that noradrenaline produces an enhancement in the activity of voltage-dependent calcium channels in granule cells of the hippocampal dentate gyrus. This action appears to be mediated by β -adrenoceptors and can be mimicked by cyclic AMP.

The preparation consisted of acutely exposed granule cells from guinea-pig hippocampal slices (Fig. 1a). The techniques used for the proteolytic treatment of slices to expose granule cells and pyramidal neurons are given elsewhere¹⁵. The somata of granule cells were voltage or current clamped in the whole-cell configuration, or patch clamped in the cell-attached configuration using standard techniques¹⁶. Pipette and bath solutions were formulated to block all potassium and sodium currents, and drugs were applied by pressure ejection through a single- or double-barrelled puffer pipette (tip diameter $\sim 2 \mu\text{m}$) placed near the soma of the clamped cell. Quantitative data were taken from 45 complete experiments—33 whole-cell and 12 cell-attached patch recordings. Partial observations were made in an additional 163 experiments.

In whole-cell recordings, step depolarizations, from a holding potential of -60 mV to potentials of -35 mV and more positive, activated an inward current whose peak amplitude was a function of the command potential (Fig. 1b). It was inferred that

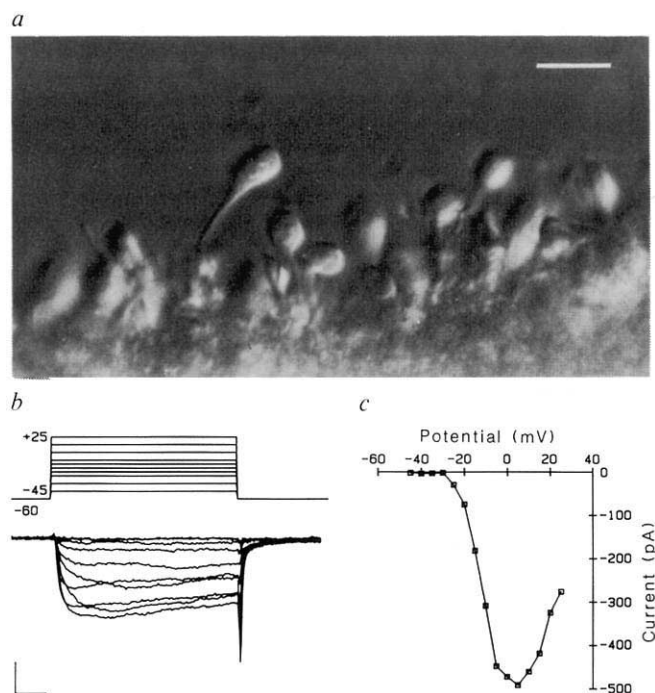


Fig. 1 Exposed granule cells and whole-cell Ca currents. *a*, Photomicrograph of area dentata, showing exposed granule cell somata. Scale bar, $20 \mu\text{m}$. *b*, Voltage commands (top) and associated inward currents (bottom). Scale bar, 200 pA vertical; 5 ms horizontal. *c*, Plot of peak inward current against command potential.

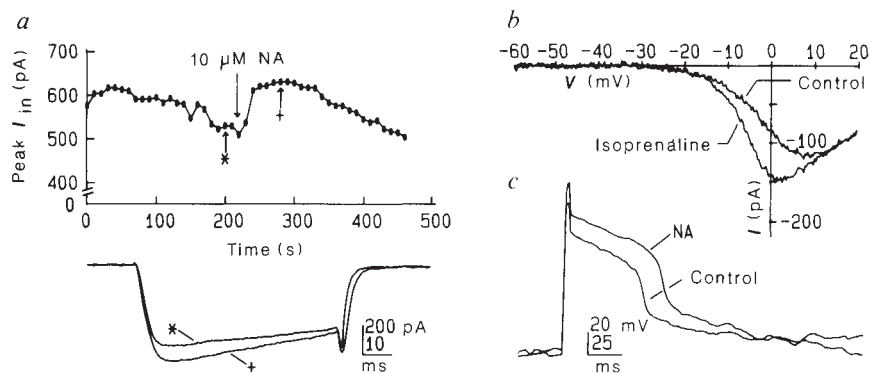
Methods. The extracellular, or bath, saline normally contained (in mM): NaCl, 149; KCl, 5; MgCl_2 , 1; dextrose, 10; HEPES, 10; CaCl_2 , 2. Tetrodotoxin (TTX) ($1 \mu\text{M}$) and 3,4-diaminopyridine (3,4-DAP) ($100 \mu\text{M}$) were added at the time of the experiment. Where indicated, 2 mM BaCl_2 was substituted for CaCl_2 . The cytoplasmic saline in early experiments contained (in mM): potassium acetate, 90; CsCl, 50; HEPES, 10; EGTA, 5; 3,4-DAP, 0.1. In later experiments potassium acetate was replaced by caesium acetate, and in some experiments Mg-ATP (5 mM) was added to the cytoplasmic saline. The pH of bath and cytoplasmic salines was adjusted to 7.35 with tetraethylammonium hydroxide (TEA-OH), with final TEA concentrations of about 6 and 15 mM , respectively. The linear leakage and uncanceled capacity currents were digitally subtracted from all current traces shown by adding an averaged and scaled hyperpolarizing transient to the raw data.

this inward current is carried by Ca^{2+} ions, because it could be totally eliminated by adding Cd (0.1 mM) or Ni (0.5 mM) to the bath. A plot of the peak inward Ca current against command potential for one experiment is shown in Fig. 1c. The maximum inward current in this and other experiments was usually reached with commands to $\sim 0 \text{ mV}$.

The initial protocol used to test for effects of noradrenaline on whole-cell Ca currents involved clamping the cell at -60 mV and applying a step command to 0 mV every 7–15 s. After the baseline Ca current had reached a relatively stable level, a pressure pulse was applied to the noradrenaline-containing pipette (see Fig. 2a). Approximately 20 s after the ejection of noradrenaline, there was an increase in the amplitude of the peak inward current. Similar results were obtained with the β -adrenoceptor agonist isoprenaline (average increase $37 \pm 11\%$ (mean $\pm$ s.e.m), $n = 10$), whereas clonidine ($2 \mu\text{M}$), an α_2 -adrenoceptor agonist, did not increase the inward current ($n = 3$). A biologically inactive enantiomer of isoprenaline, (+)-isoprenaline at $1 \mu\text{M}$, had no effect on the calcium current ($n = 3$). To assess the voltage range of the increased inward current, a slow ramp voltage command was used before, and 30 s after, applying isoprenaline (see Fig. 2b). In these experi-

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Fig. 2 Application of noradrenaline or isoprenaline increases the peak inward current. *a*, Plot of the peak inward current against time after formation of the whole-cell clamp (above). *, +, Sampling stages shown in detail below. A 500-ms pressure pulse was applied to a puffer pipette containing 10 μ M noradrenaline (NA) at the time indicated by the downward arrow. Below, sample traces before (*) and after (+) drug application. The charge carrier was Ca. Holding potential, -60 mV; command potential, 0 mV. *b*, Currents elicited by a ramp voltage command from -60 to +20 mV (200 ms duration) in control saline and after a 500-ms puff of 2 μ M isoprenaline. Charge carrier was Ba. *c*, Effect of application of 10 μ M noradrenaline (NA) on probable Ca-dependent action potentials. A 500-ms puff of noradrenaline was applied to the cell while in the current-clamp mode. Regenerative spikes were elicited by brief (2 ms) injections of outward current before and 14 s after applying noradrenaline. Internal (caesium acetate/CsCl) and external solutions were as in Fig. 1 legend.



ments, Ba^{2+} was used in place of Ca^{2+} in the bath saline to reduce inactivation during the ramp. Isoprenaline increased Ba currents over a wide voltage range (Fig. 2*b*). The apparent shift of the peak current to more negative potentials after isoprenaline treatment may reflect residual inactivation of the Ba current, a voltage-dependent enhancement of the Ba current¹⁷, altered channel kinetics¹⁷, or selective actions of isoprenaline on one or more multiple channel types with different voltage dependencies^{18,19}.

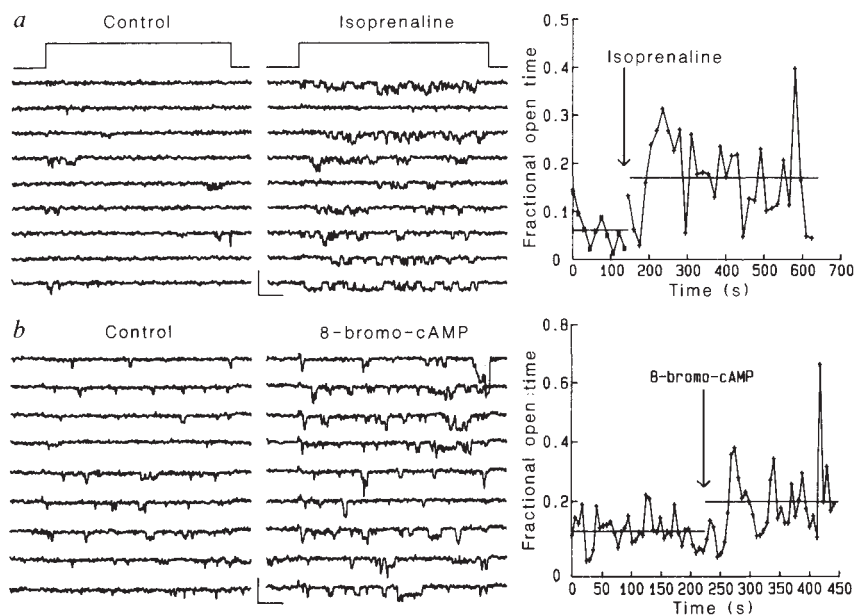
In other experiments, the effects of noradrenaline were examined on Ca-dependent action potentials evoked by brief current pulses under conditions in which all potassium currents were blocked (Fig. 2*c*). Noradrenaline produced an increase in the amplitude and duration of the Ca spikes. Qualitatively similar increases were seen in four experiments.

Activation of β adrenoceptors has been shown, in the hippocampus and elsewhere, to produce an increase in cAMP by activation of adenylyl cyclase^{20,21}. If the increase in Ca current observed with β -adrenoceptor agonists is mediated by intracel-

lular cAMP, then directly increasing cAMP should have similar effects. To test for this hypothetical mechanism, we applied a brief pressure pulse to a pipette containing the diterpene compound forskolin, which directly activates adenylyl cyclase, in a protocol similar to that of Fig. 2*a*. Forskolin was found to mimic qualitatively the effects of noradrenaline and isoprenaline in increasing Ca currents (average increase $18 \pm 3\%$, $n = 7$). Similar application of the membrane-permeable derivative 8-bromo-cAMP, at 2–5 mM, also increased the inward Ca current (average increase $44 \pm 23\%$, $n = 7$).

The whole-cell calcium current, I , can be described by $I = iNP_{\text{open}}(t)$ where i is the single-channel current; N is the number of channels and $P_{\text{open}}(t)$ is the average opening probability of the channels. Therefore, an increase in I must be due to an increase in one or more of the parameters N , P and i . To explore these possibilities we made cell-attached patch-clamp recordings of Ba current through single Ca channels. Channel activity (NP) was estimated before and after application of 2 μ M isoprenaline by measuring the fraction of time during each command that

Fig. 3 *a*, Application of isoprenaline increases the activity of Ca channels in a cell-attached patch from a granule cell. Patch pipettes contained 96 mM BaCl_2 ; 100 μ M 3,4-DAP; 1 μ M TTX; and 10 mM HEPES; the pH was adjusted to 7.35 with TEA-OH. The exposed cells were bathed in 140 mM potassium-aspartate; 20 mM dextrose; 1 mM MgCl_2 ; 10 mM EGTA; 10 mM HEPES. The pH was adjusted to 7.4 with KOH. The potassium aspartate saline was used to zero the membrane potential across the whole cell (see Nowycky *et al.*¹⁸). Voltage jumps were applied to the membrane patch through the patch electrode, and potentials given refer to the patch membrane potential. Left, consecutive traces of channel activity in control saline in response to step commands from -100 to -10 mV. Centre, channel activity in the same patch 95 s after a 500-ms pressure pulse was applied to a puffer pipette that was located near the cell body and contained potassium aspartate saline plus 2 μ M isoprenaline. Right, plot of the fraction of time during the command when the current level was more negative than a threshold level (-0.3 pA) before and after application of isoprenaline. The threshold was set at -3 times the standard deviation of the baseline noise calculated from traces in which no channel activity was visible. Each plotted point is the average of three traces, and the horizontal lines indicate the means for all traces before and after drug application. Current traces shown were filtered at 2 kHz (-3 dB, Bessel response) and sampled at 10 kHz. Calibration bar: 2 pA vertical, 10 ms horizontal. *b*, Experiment in which 5 mM 8-bromo-cAMP was applied in a manner similar to that in *a*.



Each plotted point is the average of three traces, and the horizontal lines indicate the means for all traces before and after drug application. Current traces shown were filtered at 2 kHz (-3 dB, Bessel response) and sampled at 10 kHz. Calibration bar: 2 pA vertical, 10 ms horizontal. *b*, Experiment in which 5 mM 8-bromo-cAMP was applied in a manner similar to that in *a*.

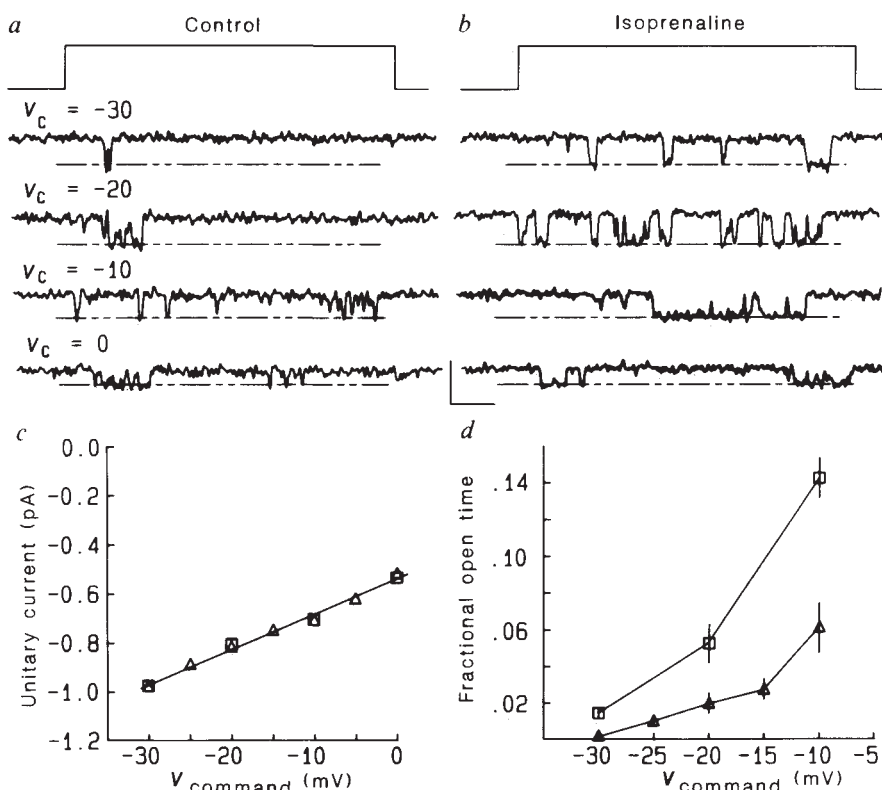


Fig. 4 Single-channel activity at different command voltages before and after isoprenaline addition. The holding voltage was -100 mV. Patch voltages are relative to the cell potential, which was near zero in potassium aspartate saline. Data are from the same patch as in Fig. 3a. *a*, Sample traces are shown for each of four different command voltages. Dashed line, major current level at each command voltage. These data were filtered at 1 kHz. *b*, Same as *a*, but 400–800 s after a 500-ms pressure pulse was applied to a puffer pipette containing potassium aspartate saline plus $2 \mu\text{M}$ isoprenaline. Calibration bar: 1 pA vertical, 10 ms horizontal. *c*, Plot of major single-channel current levels against patch potential before ($\triangle$) and after ($\square$) applying isoprenaline. The slope conductance was 14.5 pS and did not change after isoprenaline application. *d*, Plot of fractional open time against patch potential before ($\triangle$) and after ($\square$) isoprenaline application.

the inward current was greater than the baseline noise level. The mean fractional open time increased from 0.061 ± 0.013 (s.e.m) during 30 control commands to 0.170 ± 0.015 during 100 commands following application of isoprenaline (Fig. 3a). With isoprenaline, the average increase in fractional open time was $206 \pm 14\%$ ($n = 3$). Similar increases in channel activity were observed with noradrenaline (average increase $441 \pm 351\%$, $n = 3$) and 8-bromo-cAMP (Fig. 3b) (average increase $81 \pm 10\%$, $n = 6$).

In patches in which the membrane potential was held at different levels, we saw no evidence of changes in single-channel conductance (Fig. 4). In Fig. 4 an experiment is shown in which a 14.5 pS channel, which may be similar to the N type described by Nowycky *et al.*^{18,19}, was present in the recordings. No change in the conductance occurred after isoprenaline application, although the fractional open time increased at each of the voltage levels tested.

In summary, we find that noradrenaline and the β -adrenoceptor agonist isoprenaline increase voltage-dependent calcium currents through an increase in the activity of at least one type of voltage-dependent Ca channel in acutely exposed hippocampal granule neurons. This action can be mimicked by the activation of adenylyl cyclase with forskolin or by the direct application of a membrane-permeable analogue of cAMP, supporting the hypothesis that the intracellular second messenger cAMP is responsible, in part, for the changes in channel properties. The observed increase in channel activity could be due to an increased open time of the individual channels or an increase in the probability of opening of the same or other channels.

In hippocampal pyramidal neurons, noradrenaline has also been reported to decrease a Ca-activated potassium conductance^{22,23}. Such a decrease in potassium conductance may also take place in hippocampal granule neurons, but this would not have been observed in our experiments because all potassium channels were blocked with ion substitution and drugs.

Noradrenergic modulation of neuronal Ca currents could exert important effects on cerebral function. Although the mechanism underlying LTP is unclear, one important step in the process appears to be postsynaptic Ca influx^{24,25}. Previous

results indicating that noradrenaline has a modulatory effect on LTP^{12,26}, combined with the results reported here and elsewhere^{22,23,27}, suggest that noradrenaline may enhance LTP by increasing Ca influx into postsynaptic elements. The noradrenaline enhancement of Ca influx would occur only under conditions in which the membrane potential was sufficiently depolarized to activate Ca channels and thus might provide a plausible explanation for some of the reported frequency- or activity-dependent actions of the neurotransmitter¹².

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Molecular distinction between muscarinic acetylcholine receptor subtypes

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The muscarinic acetylcholine receptor (mAChR) mediates various cellular responses, including inhibition of adenylate cyclase, breakdown of phosphoinositides and modulation of potassium channels, through the action of guanine nucleotide-binding regulatory proteins¹⁻³. Pharmacologically distinguishable forms of the mAChR occur in different tissues and have provisionally been classified into M₁ (I), M₂ cardiac (II) and M₂ glandular (III) subtypes on the basis of their difference in apparent affinity for antagonists⁴⁻⁸. In an attempt to elucidate the molecular basis of the functional heterogeneity of the mAChR, we have cloned and sequenced DNAs complementary to porcine cerebral and cardiac messenger RNAs encoding mAChRs and have thereby deduced the primary structures of the receptor proteins^{9,10}. We report here that the messenger RNA generated by transcription of the cardiac complementary DNA directs the formation of a functional mAChR in *Xenopus* oocytes and that this mAChR differs from the mAChR formed by expression of the cerebral cDNA⁹ both in acetylcholine (ACh)-induced response and in antagonist binding properties. Our results provide evidence indicating that the mAChR encoded by the cerebral cDNA (designated as mAChR I) and the mAChR encoded by the cardiac cDNA (mAChR II) are of the M₁ (I) and the M₂ cardiac (II) subtype, respectively.

The cloned cDNA encoding mAChR II (ref. 10), including the poly(dA) tract, was linked with the bacteriophage SP6 promoter¹¹ and transcribed *in vitro* with SP6 polymerase. The mAChR II-specific mRNA thus obtained was injected into *Xenopus* oocytes, which were then examined for functional properties in comparison with oocytes injected with the mAChR I-specific mRNA. The (-)[³H]N-methylscopolamine (NMS) binding activity on the cell surface as well as the (-)[³H]quinuclidinyl benzilate (QNB) binding activity in the cell extract was much higher for oocytes injected with the mAChR II-specific mRNA than for oocytes injected with the mAChR I-specific mRNA (Table 1). The reason for this difference is not known.

All the oocytes injected with the mAChR II-specific mRNA responded to 1 μ M ACh, the peak inward current being 83 $\pm$ 38 nA (mean $\pm$ s.d., n = 30) at -70 mV membrane potential. A typical response to ACh observed in oocytes implanted with mAChR II comprised an initial smooth inward current followed by an oscillatory component (Fig. 1A, a). Application of hyperpolarizing test pulses indicated that these inward currents were accompanied by increased membrane conductance. The oscillatory current varied in amplitude among the oocytes tested and was undetected in some of them. Carbamylcholine, muscarine, pilocarpine and arecoline (1 μ M each) induced a similar response. Atropine and (-)scopolamine (0.1 μ M each) abolished the ACh response in a reversible manner, whereas (+)tubocurarine (2.5 μ M) and α -bungarotoxin (0.1 μ M) exerted no effect.

The nature of the ACh response observed in oocytes injected with the mAChR II-specific mRNA differed from that observed in oocytes injected with the mAChR I-specific mRNA. The average peak inward current measured in oocytes implanted with mAChR II was much smaller than that measured in oocytes implanted with mAChR I (Table 1). The difference was 560-fold when the density of mAChRs expressed on the cell surface, estimated by (-)[³H]NMS binding, was taken into account

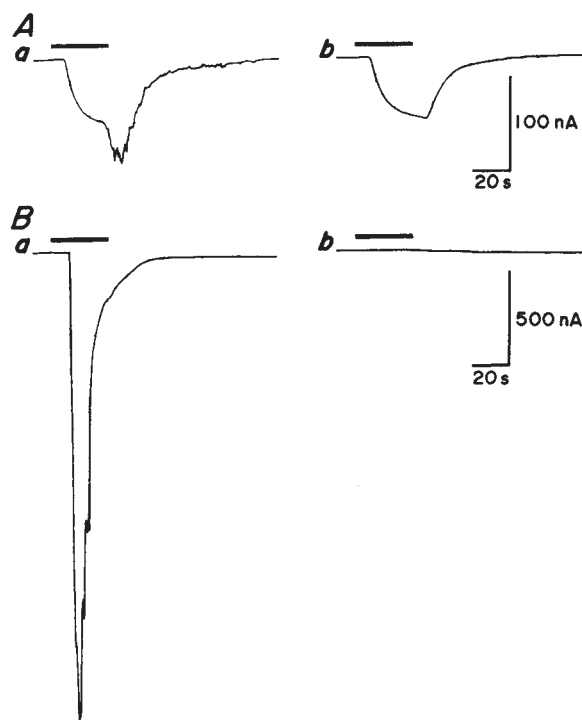


Fig. 1 Effect of EGTA on ACh-activated currents in *Xenopus* oocytes injected with the mAChR II-specific mRNA (A) or with the mAChR I-specific mRNA (B). Whole-cell currents activated by bath application of 1 μ M ACh were recorded under voltage clamp at -70 mV membrane potential before (a) and after (b) intracellular injection of EGTA. Inward current is downward. The duration of ACh application is indicated by bars without taking into account the dead-space time in the perfusion system ($\sim$ 7 s). EGTA was injected ionophoretically into the oocytes using a micropipette filled with 100 mM EGTA adjusted to pH 8.0 with KOH (intensity, 1 μ A; duration, 6 min). After treatment with EGTA, no detectable current oscillations were observed in oocytes injected with either mRNA even when ACh application was prolonged to 2 min.

(Table 1). The latency of the ACh response observed in oocytes implanted with mAChR II (Fig. 1A, a) was shorter than that observed in oocytes implanted with mAChR I (Fig. 1B, a). Most of the latency of the ACh response in mAChR II-implanted oocytes was due to the dead-space time in the perfusion system ($\sim$ 7 s), whereas the current response in mAChR I-implanted oocytes occurred after an additional delay (3 ± 2 s, n = 30) in agreement with our previous report⁹. The current evoked by activation of mAChR I is oscillatory in nature and is caused principally by an increase in the chloride conductance of the oocyte membrane, as reported previously⁹. It is known that an elevation in intracellular calcium concentration leads to activation of chloride channels in *Xenopus* oocytes¹²⁻¹⁴. Replacement of extracellular Ca²⁺ by Mg²⁺ did not significantly affect the ACh-activated oscillatory current in oocytes implanted either with mAChR I or with mAChR II. But intracellular injection of the calcium-chelating agent EGTA almost completely abolished the ACh-activated current in oocytes implanted with mAChR I (Fig. 1B, b), leaving only a small long-lasting inward current (31 ± 17 nA in amplitude and 8 ± 2 min in duration, n = 17, no detectable current being observed in three of the 20 oocytes tested). The oscillatory current in oocytes implanted with mAChR II similarly disappeared after this treatment, whereas the smooth component was virtually unaffected (Fig. 1A, b). These results indicate that activation of mAChR II leads to the opening of at least two distinct classes of ionic channels in *Xenopus* oocytes.

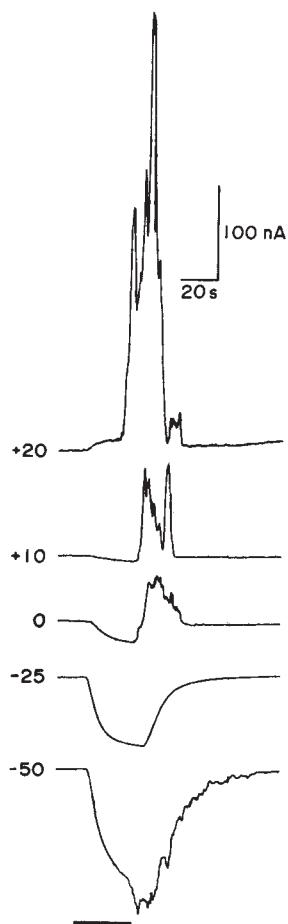


Fig. 2 Membrane-potential dependence of ACh-activated currents in a *Xenopus* oocyte injected with the mAChR II-specific mRNA. The oocyte was incubated for two days before being tested. Whole-cell currents activated by bath application of $1 \mu\text{M}$ ACh were recorded under voltage clamp at different membrane potentials (mV) as indicated on the left-hand side of each trace. Inward current is downward. The duration of ACh application is indicated as in Fig. 1.

Figure 2 shows the ACh-activated currents recorded from an oocyte implanted with mAChR II at various membrane potentials. The oscillatory current was reversed in polarity at a potential around -25 mV , which is close to the equilibrium potential of chloride ions in *Xenopus* oocytes^{12,15,16}. In contrast, the reversal potential of the smooth current was around 10 mV . This value does not correspond to the equilibrium potential of any single species of ions, indicating the involvement of more than one ion species in the generation of the smooth current. The ionic basis of the smooth current was further examined in EGTA-loaded oocytes. The reversal potential in Ringer's solution was $11 \pm 3 \text{ mV}$ ($n = 10$) and was virtually unchanged even when external Cl^- was completely replaced by SO_4^{2-} . In a K^+ -free solution (120 mM Na^+), the reversal potential was $11 \pm 1 \text{ mV}$ ($n = 5$) and shifted to $-2 \pm 1 \text{ mV}$ ($n = 5$) when the Na^+ concentration was reduced to one half by substitution with Tris. The reversal potential in a Na^+ -free solution (120 mM K^+) was $-2 \pm 1 \text{ mV}$ ($n = 5$) and changed to $-15 \pm 1 \text{ mV}$ ($n = 5$) when the K^+ concentration was halved by substitution with Tris. These results suggest that the smooth current evoked by activation of mAChR II is carried principally by sodium and potassium ions, whereas the oscillatory current is carried mainly by chloride ions.

The antagonist binding properties of mAChR II were compared with those of mAChR I using extracts from oocytes injected with the respective mRNAs (Fig. 3 and Table 1). The

Table 1 Functional properties of mAChR I and mAChR II expressed in *Xenopus* oocytes

	mAChR I	mAChR II
(-)[^3H]NMS binding on cell surface (fmol per oocyte)	2 ± 0.8 (4)	67 ± 15 (4)
(-)[^3H]QNB binding in cell extract (fmol per oocyte)	2 ± 0.4 (4)	101 ± 34 (4)
K_D (M)		
(-)[^3H]QNB	7.8×10^{-11}	8.1×10^{-11}
atropine	4.4×10^{-10}	1.5×10^{-9}
pirenzepine	1.0×10^{-8}	5.0×10^{-7}
AF-DX 116	1.8×10^{-6}	3.6×10^{-7}
ACh-activated current (nA)	$1,390 \pm 750$ (30)	83 ± 38 (30)

Data are given as means $\pm$ s.d. when indicated; numbers in parentheses refer to the number of experiments for ligand binding activity or the number of oocytes for current measurements. The apparent K_D for (-)[^3H]QNB was measured by Scatchard analysis, and those for atropine, pirenzepine and AF-DX 116 were calculated²¹ from the IC_{50} values obtained from Fig. 3. Whole-cell currents activated by $1 \mu\text{M}$ ACh (bath-applied for 30 s) were recorded under voltage clamp at -70 mV membrane potential. Our detectable limits were $\sim 0.8 \text{ fmol per oocyte}$ for (-)[^3H]NMS binding activity, $\sim 0.1 \text{ fmol per oocyte}$ for (-)[^3H]QNB binding activity and $\sim 10 \text{ nA}$ for ACh-activated current. Control oocytes (injected with water or noninjected) exhibited neither (-)[^3H]QNB binding nor ACh-activated current; the absence of the endogenous mAChR^{15,16} may result from seasonal or individual variations^{16,22}.

Methods. The recombinant plasmids used for the synthesis of the mAChR-specific mRNAs were constructed as follows. The HindIII(-92)/BstXI(415) fragment from clone phmACR905 (ref. 10), the BstXI(415)/HinfI(854) fragment from clone pmACR423 (ref. 10), the ~ 1.4 -kilobase (kb)-pair HinfI(854)/PstI (in the 3'-noncoding sequence) fragment from clone phmACR15 (ref. 10) and the 2,986-base-pair (bp) PstI/HindIII fragment from the plasmid pSP64 (ref. 11) were ligated to yield the plasmid pSPHM10; numbers in parentheses indicate the 5'-terminal nucleotide generated by cleavage. The ~ 3.9 -kb-pair BglII/PstI fragment from pSPHM10, the ~ 1.5 -kb-pair PstI (in the 3'-noncoding sequence)/RsaI (on vector) fragment from phmACR15 and the 1,397-bp HincII/BglI fragment from pSP64 were ligated to yield the plasmid pSPHM45. The plasmid pSPHM10 (ref. 9) was cleaved by EcoRI, treated with T4 DNA polymerase in the presence of the four deoxyribonucleoside triphosphates, ligated with the synthetic HindIII linker 5'-ACAAGCTTGT-3' and cleaved by HindIII and BglII. The resulting 1,833-bp fragment was ligated with the ~ 890 -bp BglII(1,656)/PvuII (on vector) fragment from clone pmACR84 (ref. 9) and the 2,982-bp HincII/HindIII fragment from pSP64 to yield the plasmid pSPBM1. The mAChR II-specific and the mAChR I-specific mRNAs were synthesized by transcription *in vitro*^{11,23} using XbaI-cleaved pSPHM45 and pSPBM1 as templates, respectively. The resulting mRNAs were shown by electrophoresis on 1.5% agarose gel to be homogeneous and to have expected sizes of $\sim 3.9 \text{ kb}$ (mAChR II-specific mRNA) and $\sim 2.7 \text{ kb}$ (mAChR I-specific mRNA). Each of the mRNAs was injected into *Xenopus laevis* oocytes (mRNA concentration, $0.4 \mu\text{g } \mu\text{l}^{-1}$; average volume injected per oocyte, $\sim 40 \text{ nl}$). Before being tested, the injected oocytes were incubated in the medium described previously⁹ at 19°C for three days unless otherwise indicated. (-)[^3H]NMS binding activity on the cell surface was estimated as described previously²⁴, except that oocytes were incubated with 5 nM (-)[^3H]NMS (72 Ci mmol^{-1}) for 2 h. (-)[^3H]QNB binding activity in the cell extract was measured as described previously⁹, except that 1 nM (-)[^3H]QNB ($42.6 \text{ Ci mmol}^{-1}$) was used. Electrophysiological measurements were carried out at 19 – 23°C in Ringer's solution under the conditions described previously⁹ unless otherwise specified; the Na^+ -free solution was composed of 120 mM KCl , 1.8 mM CaCl_2 and 10 mM Tris-HCl ($\text{pH } 7.2$), and the K^+ -free solution of 120 mM NaCl , 1.8 mM CaCl_2 and 10 mM Tris-HCl ($\text{pH } 7.2$).

apparent dissociation constant (K_D) for (-)[^3H]QNB was similar for mAChR II ($81 \pm 43 \text{ pM}$, $n = 3$) and mAChR I ($78 \pm 29 \text{ pM}$, $n = 3$). The apparent K_D values for atropine, pirenzepine (selective for the M_1 subtype) and AF-DX 116 (selective for the

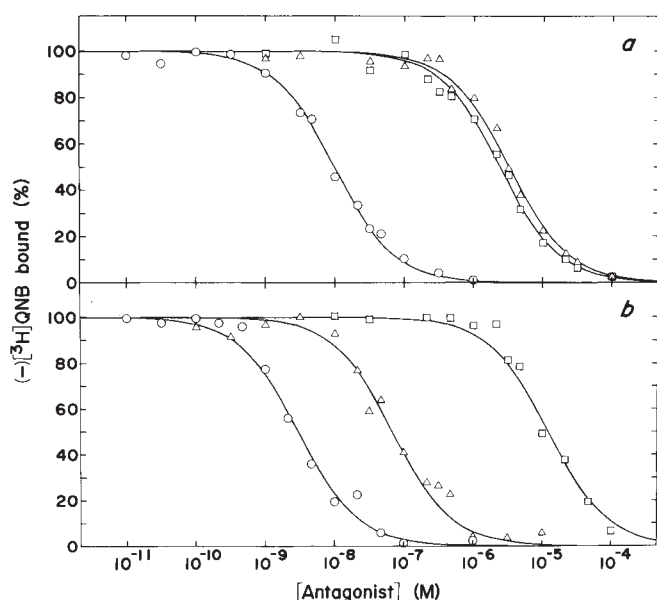


Fig. 3 Effects of atropine (○), pirenzepine (△) and AF-DX 116 (□) on (–)[³H]QNB binding in extracts from *Xenopus* oocytes injected with the mAChR II-specific mRNA (a) or with the mAChR I-specific mRNA (b). Cell extracts were prepared and displacement of (–)[³H]QNB binding by unlabelled antagonists measured as described previously⁹, except that 0.44 nM (–)[³H]QNB was used and that values for 0% binding were determined by measurements in the presence of 10 μM atropine; the 0% values (158–281 d.p.m. (a) or 105–232 d.p.m. (b)) were 2–8% (a) or 19–34% (b) of the 100% values. Each point represents the mean from 2–5 separate experiments. The theoretical curves have been drawn by nonlinear least-squares analysis according to the equation $y = (1 + x/IC_{50})^{-1}$, where x represents the antagonist concentration. The IC_{50} values for atropine, pirenzepine and AF-DX 116 were 9.8 nM, 3.2 μM and 2.3 μM (a) and 3.0 nM, 69 nM and 12 μM (b), respectively.

M_2 cardiac subtype; ref. 6) were obtained by measuring displacement of (–)[³H]QNB binding. Only a small difference was found in the K_D values for atropine of mAChR II (1.5 nM) and mAChR I (0.44 nM), in agreement with previous reports^{17–19}. In contrast, pirenzepine clearly discriminated between the two mAChRs. The K_D for pirenzepine of mAChR II was 500 nM, being 50-fold larger than that of mAChR I (10 nM). These values compare well with the reported K_D for the M_2 cardiac and the M_1 subtype of the mAChR, respectively^{4,17–20}. Furthermore, mAChR II showed a higher binding affinity for AF-DX 116 ($K_D = 0.36$ μM) than mAChR I ($K_D = 1.8$ μM). This is again in agreement with the finding that the affinity for AF-DX 116 of the M_2 cardiac subtype is 5- to 7-fold higher than that of the M_1 subtype^{6,18}; the M_2 glandular subtype has ~4-fold lower affinity for AF-DX 116 than the M_1 subtype⁶.

The antagonist binding properties of mAChR I and mAChR II, together with the different ACh responses mediated by them, provide evidence indicating that mAChR I and mAChR II represent the M_1 (I) and the M_2 cardiac (II) subtype, respectively. Thus, these subtypes of the mAChR are distinct gene products. In accord with this conclusion are our previous results of RNA blot hybridization analysis, which show that the mAChR I mRNA is present predominantly in the cerebral cortex and corpus striatum, whereas the mAChR II mRNA prevails in the heart and medulla-pons^{9,10}. The different ACh responses elicited by mAChR I and mAChR II may imply that these mAChR species are linked with distinct muscarinic effects, although the responses observed in *Xenopus* oocytes may not necessarily correspond to those mediated by the native mAChRs in mammalian cells.

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Identification and spatial distribution of retinoids in the developing chick limb bud

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All-trans-retinoic acid (RA) induces striking digit pattern duplications when locally applied to the developing chick limb bud^{1–4}. Instead of the normal digit pattern (234) a mirror-symmetrical 432234 pattern can be specified. Hence, RA closely mimics posterior limb bud tissue (the zone of polarizing activity, ZPA) that causes very similar duplications when grafted to an anterior site of a host limb bud⁵. This resemblance raises an intriguing possibility: that RA is related to the as yet unidentified inducer substance thought to be released by the ZPA^{1,6}. Here we report that chick limb buds contain endogenous RA and we show that RA, but not its biosynthetic precursor retinol, forms a concentration gradient across the limb anlage with a high-point in the posterior domain of the limb bud, the part that also contains the ZPA.

To search for endogenous all-trans-retinoic acid 500 to 800 fore- and hindlimb buds were dissected from stage-21 embryos⁷ (3.75 d). Only such early buds are capable of responding to ZPA grafts or RA administration^{2,6,8}. Before disruption, 1.8 ± 0.04 ng of ³H-RA (internal standard⁹) was added to the tissue, followed by extraction and fractionation by reverse-phase HPLC (Fig. 1 legend). Peak A₂ coelutes with authentic ³H-RA (Fig. 1a). Fractions containing peak A₂ were rechromatographed through either a normal phase HPLC or a C₁₈ column with a different solvent. In the first instance a chromatogram with a distinct peak (B in Fig. 1b) is obtained. Peak B coelutes with internal standard and has an OD₃₅₀/OD₂₈₀ ratio of about 5, typical for RA. Reverse-phase analysis of A₂ again reveals one major peak (C in Fig. 1c) that comigrates with the internal reference. To

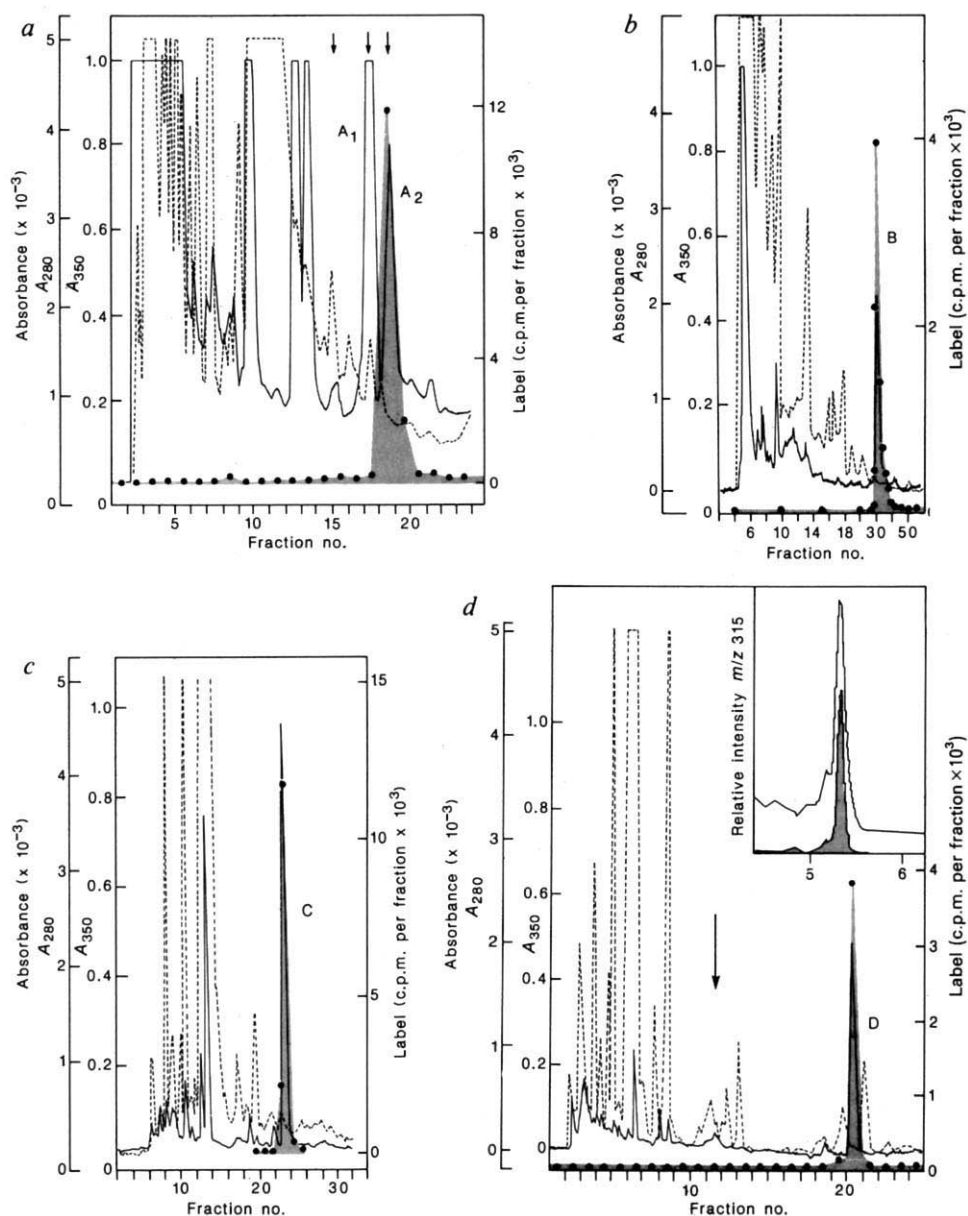
Fig. 1 HPLC sample chromatograms presented illustrate the purification and identification of all-*trans*-retinoic acid (RA) from stage-21 chick limb buds. Broken lines, absorbance at 280 nm; solid lines, absorbance at 350 nm; shaded areas with black dots, elution profiles of the internal standard ^3H -RA determined by scintillation counting. *a*, Fractionation of extracts of limb buds on a C_{18} column (solvent A at a flow rate of 1.2 ml min^{-1}) results in two peaks (A_1 and A_2) that absorb strongly at 350 nm but weakly at 280 nm (typical for retinoids). A_2 coelutes with ^3H -RA. Arrows, elution position of external standards 13-*cis*-retinoic acid, all-*trans*-retinol and all-*trans*-retinoic acid (left to right). *b*, Further purification of peak A_2 by normal phase HPLC (solvent B, 2 ml min^{-1}) results in peak B that coincides with authentic ^3H -RA internal standard. *c*, Repurification of peak A_2 by reversed phase HPLC (solvent C, 1.2 ml min^{-1}) reveals that the unknown coelutes with authentic ^3H -RA. *d*, Co-purification of the methyl ester of peak C and authentic ^3H -all-*trans*-retinoic acid methyl ester on a C_{18} column, developed with solvent C (1.2 ml min^{-1}). Former peak C has completely vanished, demonstrating that it represented pure RA. Arrow, elution position of external standard RA. Inset, ammonia chemical ionization GC/MS selected ion monitoring traces at m/z 315 (MH^+); authentic all-*trans*-retinoic acid methyl ester (lower shaded profile) and methyl ester of peak C (top) are shown; horizontal axis, retention time in min.

Methods. After dissection performed in an ice-water bath in the presence of stabilizing buffer¹¹, limb buds were extensively washed in ice cold stabilizing buffer and collected into a vial immersed in liquid nitrogen. On thawing, ethanol was added to

15% (v/v). To serve as an internal standard either $1.8 \pm 0.04 \text{ ng}$ ($\sim 20 \text{ nCi}$) of all-*trans*-[10,11- $^3\text{H}_2$]-retinoic acid (Dr S. W. Rhee, SRI International) or $240 \pm 2 \text{ pg}$ ($\sim 40 \text{ nCi}$) all-*trans*-[11,12- $^3\text{H}_2$]-retinol (Amersham) were added. The tissue ($\sim 4 \text{ ml}$) was sonicated on ice, extracted 3 times with 4 ml ethyl acetate/methyl acetate (8:1) that contained $50 \mu\text{g ml}^{-1}$ butylated hydroxytoluene. After centrifugation the organic phases were pooled, the solvent evaporated at room temperature with nitrogen gas to a volume of $\sim 100 \mu\text{l}$ and the sample chilled on ice for 30 min. The precipitate formed was removed by centrifugation and the supernatant evaporated to dryness and redissolved for HPLC analysis. For HPLC, Microsorb C_{18} and silica $4.6 \text{ mm} \times 250 \text{ mm}$ columns from Rainin Instruments were used, with the following solvent systems: A, acetonitrile/methanol/1% acetic acid (6:2:2); B, *n*-hexane/acetonitrile/acetic acid (99.5:0.4:0.1); C, methanol/acetonitrile/water/acetic acid (80:10:9:1). Fractions containing peak C were transferred into $20 \mu\text{l}$ methanol and incubated with diazomethane dissolved in ether for methylation. For GC/MS analysis 3,000 stage-21 limb buds were extracted, no internal standard was added. The extract was purified twice by HPLC on a C_{18} column eluted with solvents A and C, respectively. Peak C was methylated and dissolved in $10 \mu\text{l}$ hexane; $5 \mu\text{l}$ of this was injected through a solventless injector (Chrompak) into a $30 \text{ m} \times 0.25 \text{ mm}$ fused silica DB-1 capillary column (J+W Scientific) inserted directly into the ion source of a Finnigan 4500 mass spectrometer operating in chemical ionization mode (0.6 torr ammonia, ion source at 150°C). The temperature of injector and column were 260 and 230°C , respectively. The He carrier gas pressure was 20 p.s.i. Retinoid was quantitated as $Q = [(A/U) \times 100/R] - I$; Q is the amount of endogenous retinoid (in ng), A the integrated sample peak area, U the area per ng retinoid (from calibration standards), R the percentage recovery determined from the internal standard and I the amount (ng) of internal standard initially added.

confirm the presence of a free carboxyl group in the putative RA, the fractions that contained peak C were treated with diazomethane for methylation. When rechromatographed, both the unknown compound and the radioactive standard, now in form of ^3H -all-*trans*-methyl retinoate, undergo the same pronounced shift in retention time and elute as a single peak D

(Fig. 1d). In a separate experiment, methyl ester D was analysed by gas chromatography/mass spectroscopy (GC/MS; no standard was added). GC/MS analysis in the electron impact mode and selected ion monitoring at m/z 314 (molecular ion of methyl retinoate¹⁰) shows that methyl ester D and authentic methyl retinoate coelute (data not shown). This was further confirmed



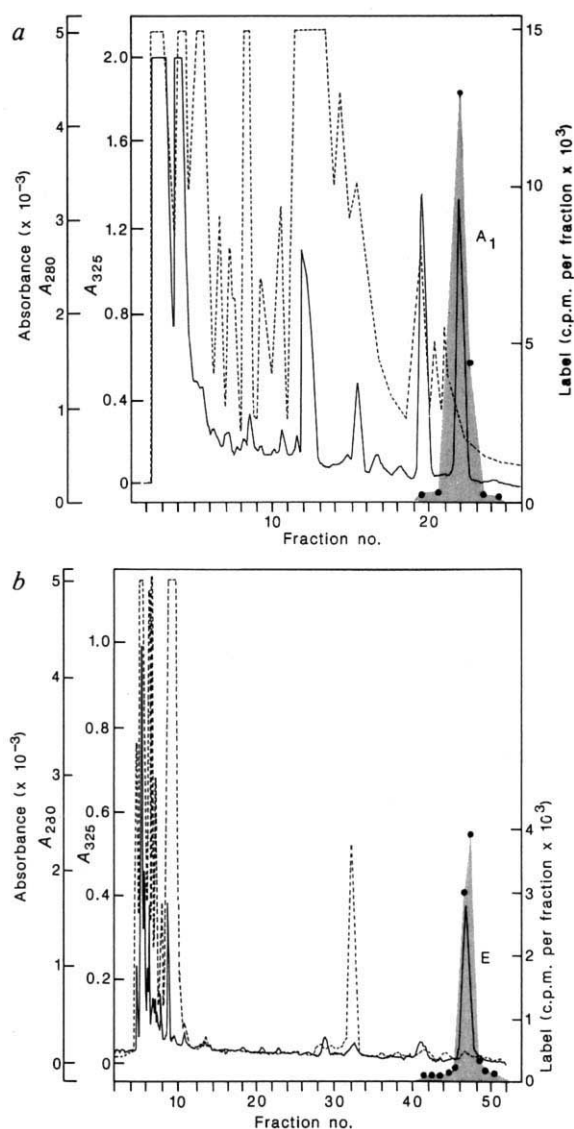


Fig. 2 Sample chromatograms illustrating the purification and identification of all-*trans*-retinol from stage-21 limb buds. Broken line, absorbance at 280 nm; solid line, absorbance at 325 nm; shaded areas with dots elution profile of the internal standard ^3H -retinol determined by scintillation counting. *a*, Fractionation of bud extracts on a C_{18} reverse phase column (solvent A, 1.2 ml min^{-1}) results in a prominent peak (A_1) that co-elutes with internal standard ^3H -retinol. *b*, Peak A_1 rechromatographed on a normal phase column (solvent D, *n*-hexane/dioxane (95:5), 2 ml min^{-1}) co-elutes with the internal standard ^3H -retinol (peak E).

by selected ion monitoring at m/z 315 (MH^+ , chemical ionization mode, Fig. 1*d* insert).

RA-dependent induction of duplications requires a mean RA concentration in the tissue of $\sim 20 \text{ nM}$ (refs 3 and 11). To quantitate endogenous RA, the area of either peak B or C was integrated (Fig. 1 legend), leading to 6.3 pg RA per limb bud at stage 21. Because such a limb bud has a volume of about $0.8 \mu\text{l}$ (ref. 12) this corresponds to a mean tissue concentration of 25 nM , close to that required to induce duplications.

Peak A_1 (Fig. 1*a*) is all-*trans*-retinol. This conclusion is based on the following experimental evidence. First, ^3H -all-*trans*-retinol, added as an internal standard before tissue extraction, comigrates with peak A_1 (Fig. 2*a*). Second, repurification on a



Fig. 3 Chick wing with a 43234 digit pattern that developed following local application of RA extracted from stage-21 chick embryos. The specimen above is the contralateral, untreated control wing (234 pattern).

Methods. RA was extracted from 600 stage-21 embryos and purified by reverse and normal phase HPLC as described for limb buds. RA (117 ng) was dissolved in $5 \mu\text{l}$ DMSO to a final concentration of $23 \mu\text{g ml}^{-1}$. Twelve AG1-X2 beads (in formate form, Bio-Rad) were soaked in this solution for 30 min. Each bead was briefly rinsed in phosphate-buffered saline and implanted at the anterior margin of a wing bud of a stage-20 embryo as previously described³. The embryo was incubated for six additional days, killed and stained with alcian green, a cartilage dye.

normal-phase HPLC column results in a single peak (E in Fig. 2*b*) that coelutes with the internal standard. Third, the UV-visible absorption and fluorescence spectra of peak E fractions and of authentic retinol are identical (spectra not shown). The area of peak E was used to quantitate all-*trans*-retinol in bud tissue (see Fig. 1 legend). We find $140 \pm 7 \text{ pg}$ per bud, corresponding to a tissue concentration of $\sim 600 \text{ nM}$, 25 times more than that of RA.

To test RA purified from embryonic chick tissue for its ability to induce pattern duplications, stage 21 embryos were extracted and the extract fractionated on a C_{18} HPLC column using eluent A followed by purification by normal phase HPLC using eluent B (internal standard was omitted). The recovered RA was dissolved in dimethylsulphoxide (DMSO) to $23 \mu\text{g ml}^{-1}$ and then locally applied to wing buds of stage-20 embryos. Duplications developed with the following digit patterns: 43234 (five cases, one shown in Fig. 3), 32234 (four cases) and 2234 (one case). In an experiment carried out in parallel, beads were soaked in the same concentration of synthetic RA. The pattern duplications obtained with beads impregnated in synthetic RA were: 4334 (two cases), 43234 (four cases), 2234 (two cases). Thus, RA extracted from chick embryos is as efficient in causing pattern duplications as its synthetic counterpart.

ZPA, the tissue that induces duplications when grafted into a host limb bud⁵, extends along the entire posterior margin of the early fore- and hindlimb bud^{13,14}. To examine whether RA is enriched in this domain, limb buds of stage-21 embryos were divided into a posterior part comprising about a quarter of the total limb bud mass and an anterior domain representing the rest. The pooled parts were extracted, purified and RA quantitated as described for intact buds. Anterior and posterior domains contain similar amounts of RA, but differ threefold in DNA content¹⁵ (Table 1). Hence, when normalizing RA to DNA it becomes very clear that posterior tissue contains 2.6 ± 0.4 times more RA than anterior tissue (last column in Table 1). The mean

Table 1 Spatial distribution of all-*trans*-retinoic acid in the chick limb bud

Experiment no.	Sample size*	DNA anterior	DNA posterior	RA anterior	RA posterior	Ratio†
1	804	1.90 µg	0.61 µg	3.54 pg	2.85 pg	2.51
2	892	1.90 µg	0.61 µg	3.53 pg	2.38 pg	2.01
3	852	2.04 µg	0.59 µg	3.51 pg	3.22 pg	3.17
4	878	1.78 µg	0.62 µg	3.07 pg	3.13 pg	2.94
5	1,108	1.78 µg	0.65 µg	3.81 pg	3.26 pg	2.35
6	1,002	2.01 µg	0.62 µg	3.67 pg	2.71 pg	2.39
Mean tissue concentration‡	—	—	—	19 nM	49 nM	—

Limb buds were dissected from stage-21 embryos, rigorously washed in cold stabilizing buffer, divided at 4 °C as previously described¹¹. The two parts were collected into separate polypropylene tubes, immersed in liquid nitrogen.

* Number of limb bud parts used.

† Calculated as: ((RA/DNA) posterior)/((RA/DNA) anterior).

‡ Because a stage-21 limb bud has 3.11 µg of DNA per µl of wet volume¹², 0.62 µg DNA (posterior) and 1.90 µg DNA (anterior) correspond to tissue volumes of 0.2 and 0.6 µl, respectively.

RA concentration in the posterior tissue is ~50 nM whereas anteriorly it amounts to ~20 nM (Table 1). This suggests that RA forms a concentration gradient across the limb bud. Normalizing RA to either protein¹⁶ or lipid¹⁷ content instead of DNA, results in an enrichment factor of 2.9 and 2.3, respectively. In contrast, retinol shows no high-point in the bud. We have found 97 ± 4 pg anteriorly and 40 ± 2 pg posteriorly. This results in a posterior/anterior ratio of 1.2 ± 0.1, at best a slight enrichment in the ZPA-containing region.

Can a 2.5-fold enrichment of RA in the posterior region be of biological significance? The concentration profile of endogenous RA was compared with that established by release of RA from an anteriorly positioned bead, presoaked in 5 µg ml⁻¹ ³H-RA. This dose leads to duplications with additional digits 2, 3 and 4 (ref. 3 and unpublished results). After incubating the embryos for 16 h, the implant was removed and the treated bud was divided into a smaller anterior and a larger posterior piece. Tissue blocks from 12 buds were pooled and the ³H-RA present was quantitated by HPLC as previously described. We found 3.3 ± 0.7 pg ³H-RA anteriorly and 2.4 ± 0.5 pg posteriorly. Normalized to the DNA content of the parts (1.3 ± 0.05 anteriorly and 3.1 ± 0.16 µg posteriorly) this gives an enrichment of RA in the anterior fragment of 3.3 ± 0.3 times. This is just slightly greater than for endogenous RA (but of course the applied and the endogenous gradient are in opposite direction). Tissue taken after 8 h of treatment displays a similar distribution, with a ratio of 3.6.

Several lines of evidence from this and earlier studies corroborate the view that in the limb RA is a local chemical mediator with morphogenic properties. First, chick limb buds contain the same amount of endogenous RA as is required in the bud tissue to induce digit pattern duplications. Unless limb bud cells respond to endogenous and applied RA very differently, it follows that endogenous RA participates in the formation of the normal digits. Second, there are striking homologies between RA and ZPA. Both induce duplications in a dose-dependent manner^{1-3,18}, display a biphasic induction process^{11,19} and their competence to induce duplications is restricted to the same early stages of limb development^{2,6,8}. Third, RA and some of its congeners have dramatic effects on the pattern of regenerating amphibian limb blastemas²⁰⁻²³. This could reflect an ancient function of retinoids which is conserved throughout vertebrate evolution. Fourth, it is intriguing that RA, in contrast to its biologically apparently inactive precursor retinol, is enriched in the ZPA-containing part of the bud. This raises the possibility that posterior tissue enzymatically synthesizes RA from the much

more abundant retinol (see refs 24-26 for this catalysis in other systems) and then secretes RA into the rest of the limb. The resulting concentration gradient could then be used directly to define cell position and hence specify the antero-posterior pattern^{6,27}. Such concentration gradients have long been implicated in pattern formation²⁸⁻³⁰, but it has been very difficult to identify the underlying molecules. Alternatively, RA could function as a local permissive agent that maintains posterior cells as ZPA^{2,3}, which then provides the actual, as yet unknown, signal. In this interpretation, RA present anteriorly has no direct function in pattern formation. At present there is no proof for one or the other mechanism, but the fact that RA forms a concentration gradient, and is not confined to the posterior tissue lends support the first interpretation. For clear-cut answers to such questions it is necessary to unravel the cellular machinery that can measure and interpret a spatially graded cue.

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Ia binding ligands and cAMP stimulate nuclear translocation of PKC in B lymphocytes

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Altered subcellular distribution and activity of protein kinase C (PKC) is associated with transmembrane signalling in a variety of systems in which receptor occupancy leads to increased hydrolysis of polyphosphoinositides. Here we report evidence that in B lymphocytes, cyclic-cAMP-generating signal transduction pathways can activate translocation of PKC from the cytosol to the nucleus. Elevated cAMP levels and translocation of PKC to the nucleus are induced by antibodies against Ia antigens in normal B lymphocytes. Further, cAMP analogues mediate the translocation of PKC to the nucleus of these cells. These findings suggest that in physiological situations, ligation of B-lymphocyte Ia molecules by helper T cells leads to increased cAMP production which in turn causes PKC translocation to the nucleus. In view of recent observations that antibodies against Ia antigens induce differentiation of B cells, we conclude that nuclear PKC may function in the regulation of gene expression.

A voluminous literature has documented the role of protein kinase C in stimulus-response coupling in a number of tissues^{1,2}

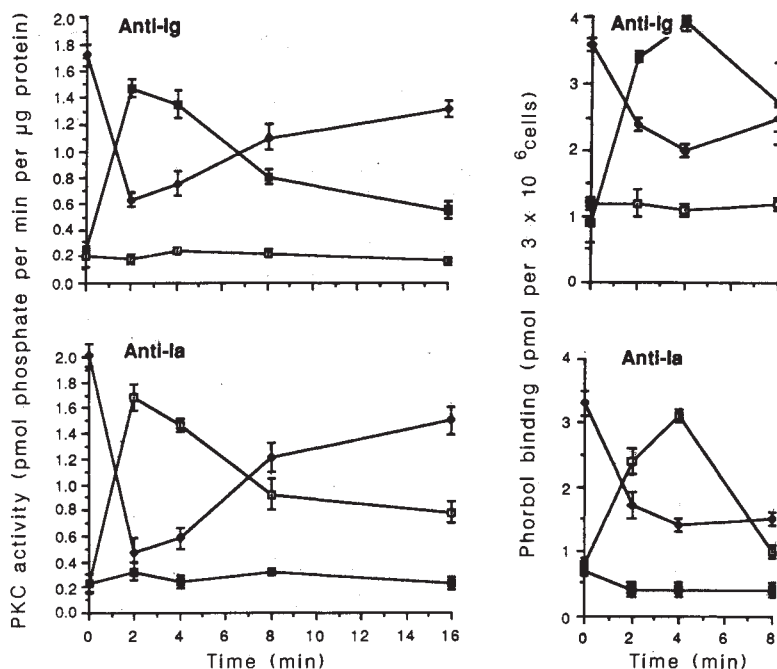
In all these systems, ligand binding to receptor initiates the hydrolysis of polyphosphoinositides by phospholipase C yielding diacylglycerol (DAG) and inositol trisphosphate (InsP₃). Diacylglycerol, phosphatidylserine (PtdSer), and Ca²⁺ which is mobilized from endoplasmic reticulum in response to InsP₃, mediate the high-affinity association of PKC, which is normally cytosolic or loosely membrane associated, with the plasma membrane^{3,4}. Subsequent PKC-mediated membrane protein phosphorylation appears to lead to the biological response.

B-lymphocyte receptors for antigen, immunoglobulins M (mIgM) and D (mIgD), transduce signals by the cascade described above⁵⁻¹⁰ leading to increased expression of *c-fos*, *c-myc* and major-histocompatibility-complex (MHC)-encoded type II molecules (Ia; I-A, I-E) and in some cases to proliferation⁸. Ligation of Ia molecules on B lymphocytes initiates signal transduction which promotes differentiation of lymphocytes into antibody secreting cells¹¹ but does not induce detectable increases in polyphosphoinositide metabolism or Ca²⁺ mobilization (J.C., unpublished observation). We recently observed¹² that antibodies against Ia antigens stimulate a transient loss in PKC activity from the cytosol of normal mouse B cells. Antibodies specific for class I molecules encoded by the MHC and expressed by B cells, do not affect PKC localization. Unlike PKC translocation which follows mIg ligation, depletion of cytosolic PKC activity mediated by antibody against Ia antigens was not mirrored by an increase in detergent-soluble membrane-associated activity. Finally, F(ab')₂ fragments of antibodies against Ia antigens have equivalent effects indicating that Fc receptors are not involved in this novel translocation response.

In view of this puzzling finding we addressed the possibility that Ia-binding ligands induce translocation of PKC to a detergent stable nuclear compartment. Quiescent B cells were stimulated with antibodies against immunoglobulin or Ia antigens for

Fig. 1 Antibodies against immunoglobulin and Ia induce qualitatively distinct PKC translocation events in B lymphocytes. Quiescent B cells were isolated as previously described¹⁰ from spleens of AKR mice and cultured (10⁷ ml⁻¹) for 0–16 min at 37 °C in balanced salt solution with no stimulus, F(ab')₂ fragments of rabbit anti-mouse immunoglobulin antibodies (10 µg ml⁻¹) or monoclonal antibodies against I-A (10.2.16, 10 µg ml⁻¹), before cytosol (◆), Triton-X-100-soluble membrane (■) and nuclear fractions (□) were isolated as previously described¹², and PKC was assayed by enzymatic activity^{10,13} (left panels) and ³H-phorbol binding¹⁴ (right panels).

Methods. Cells were cooled rapidly and washed in ice cold extraction buffer consisting of 20 mM Tris-HCl, pH 7.5, 2 mM EDTA, 50 mM 2-ME (2-mercaptoethanol) and 100 µg ml⁻¹ leupeptin. Cells were then disrupted by sonication and centrifuged at 100,000g for 1 h to yield a cytosolic supernatant and a particulate fraction. The particulate fraction was extracted with 0.1% Triton X-100 in extraction buffer and recentrifuged to generate a Triton-soluble membrane fraction (supernatant). The nuclei were prepared by extracting whole cells with 0.5% nōnidet P40 (NP40) in extraction buffer followed by centrifugation through a 10% sucrose cushion. Isolated nuclei were disrupted by sonication before analysis of PKC activity by methods previously described¹⁰. Briefly 3–5 µg (5–20 µl) cellular protein was added to 250 µl of 20 mM Tris-HCl, pH 7.5, 5 mM Mg²⁺, 0.2 mg l⁻¹ histone type III, 40 µg ml⁻¹ PtdSer, 10 µM ATP (³²P-ATP, 0.48 µCi mmol⁻¹), 500 µM Ca²⁺, 0.8 µg ml⁻¹ DAG. After incubation for 5 min at 37 °C protein was precipitated using 20% trichloroacetic acid. Precipitates were washed and ³²P incorporation determined by liquid scintillation spectrometry. Lipid-independent kinase activity was constant in all fractions, ranging from 0.2–0.3 pmol phosphate per min per µg protein, and did not change following stimulation. This activity was subtracted from values shown. Shown are the means of triplicate assays ± s.d. PKC was assayed by phorbol binding as described by Uchida and Filburn¹⁴. Cellular fractions (~30 µg protein in 50 µl) were added to 200 µl reaction mixture containing 25 mM Tris-HCl, pH 7.5, 10 mM MgAC (magnesium acetate), 1.4 mM Ca²⁺, 0.4 mM EGTA, 0.4 mM EDTA, 4 mg ml⁻¹ bovine serum albumin, 200 µg ml⁻¹ PtdSer, 30 nM [³H]-PDBU (phorbol dibutyrate), 2 µM TPA (12-O-tetradecanoyl-phorbol-13-acetate). After incubation overnight on ice, 1 ml of 20 mM Tris-HCl, pH 7.5, 10 mM MgAC, 1 mM CaCl₂ was added and cell protein was captured by filtration over 96-well Millititer HA plates (Millipore). After washing with the same buffer (5 ml per sample), filters were counted in Scintiverse II (Fisher) by liquid scintillation spectrometry. Shown are means of three assays ± s.d. Results are representative of 4–6 experiments.



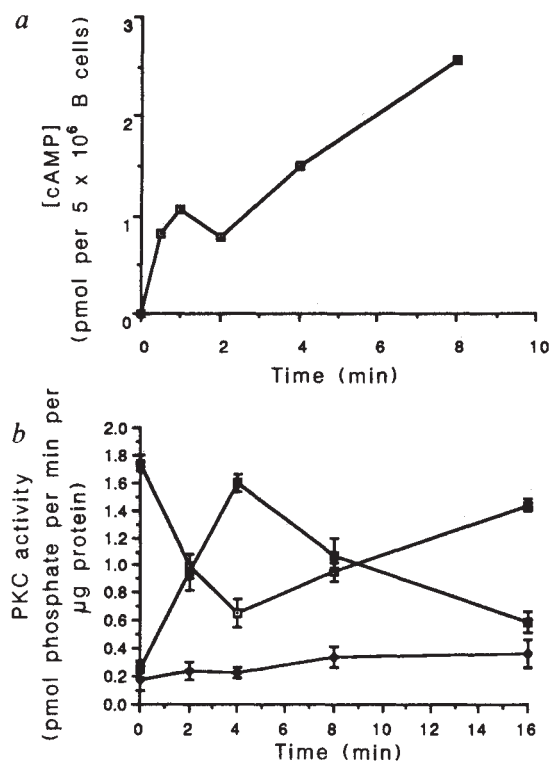


Fig. 2 Antibodies against Ia induce increases in cellular cAMP levels which, in turn induce PKC translocation in quiescent B lymphocytes. *a*, Quiescent B lymphocytes were isolated as previously described¹⁰ from spleens of AKR mice and cultured in a buffer consisting of 95 mM NaCl, 85.4 mM glucose, 10 mM HEPES, 0.1 mM EDTA and 5 mM EDTA at pH 7.4. The cells were suspended at a concentration of 5×10^6 cells ml^{-1} . Cells were cultured without stimulus or treated with F(ab')_2 fragments of anti-I-E (14-4.4S, $50 \mu\text{g ml}^{-1}$) for 30 s to 8 min at 37°C . The reaction was stopped by the addition of ice-cold trichloroacetic acid (TCA, final concentration 10%). TCA-precipitable material was removed by centrifugation at 2,000 r.p.m. for 30 min. The supernatants were removed, extracted with ether to remove the TCA, and assayed for cAMP using a commercially available ^{125}I -cAMP based radioimmunoassay³³ kit (NEN). Results are depicted as the difference in levels of cAMP (pmol per 5×10^6 cells) between stimulated and unstimulated cells at the indicated time points. Levels of cAMP in unstimulated cells (5×10^6) were: 0 time, 3.313; 30 sec, 3.269, 1 min 3.656; 2 min, 3.517; 4 min, 3.696; 8 min, 4.003. Results are representative of three experiments. *b*, Quiescent B cells were cultured in balanced salt solution containing 0.1 mM theophylline with no stimulus or 1 mM db-cAMP. At various times thereafter cells were rapidly cooled and cytosol (□), Triton-soluble membrane (◆), and nuclear fractions (■) were prepared as in Fig. 1 and analysed for PKC activity (see Fig. 1 legend). Non-PKC kinase activity, that is, activity detected in the absence of lipids, ranged from 0.2–0.3 pMol phosphate per min per μg protein. This activity did not change following cell stimulation with db-cAMP and was subtracted from the values presented. Theophylline alone had no effect on PKC levels or localization (data not shown). Shown are means of triplicate assays $\pm$ standard deviation. Results are representative of five replicate experiments.

0–16 minutes before being processed as previously described¹⁰ for analysis of cytosolic and Triton-X-100-soluble 'membrane' associated PKC, or extracted with nonionic detergent under conditions which solubilize >90% of iodinated cell-surface proteins, leaving nuclei intact. PKC activity associated with cytosolic, Triton-soluble membrane and the nuclear fraction was analysed using methods described previously^{10,13}. Antibody against immunoglobulin induced transient translocation of most functional PKC activity from the cytosol to the membrane fraction of B cells, but did not affect levels of activity associated with the nuclear fraction (Fig. 1). Antibodies against Ia induced

a loss of cytosolic activity and a concomitant increase in nuclear activity, but did not affect levels of PKC activity associated with the Triton-X-100-soluble membrane fraction. This bidirectional translocation of PKC has not been reported previously in any system.

To confirm the results of these analyses, the compartmentalization of PKC was assessed based upon ^3H -phorbol dibutyrate binding activity using the method of Uchida and Filburn¹⁴. The effects of antibodies against immunoglobulin and Ia antigens were consistent with the observations described above in that anti-immunoglobulin induced a translocation of phorbol binding activity to the membrane whereas anti-Ia induced translocation of this activity to a nuclear compartment. Therefore, by two criteria, mIg crosslinking ligands which induce phosphoinositide metabolism induce PKC translocation to the membrane, whereas mIa cross-linking ligands, which do not induce phosphoinositide metabolism induce PKC translocation to the nucleus.

We then considered the molecular basis by which nuclear translocation of PKC could occur. Although no previous evidence exists regarding the mode of signal transduction by Ia molecules, it has been known for some time that both anti-Ia and analogues of cAMP are antagonists of lymphocyte activation^{15–20}. In view of this correlation, experiments were designed to address the possibility that antibody against Ia may mediate its inhibitory effects by stimulating increases in cellular cAMP levels. Specifically, we determined whether monoclonal antibody against Ia could induce increased levels of cAMP in B cells and whether cAMP could mediate nuclear translocation of PKC in B cells. Small B cells were prepared and cultured for 0–10 min with various monoclonal anti-Ia and anti-Ig reagents or no stimulus, and subsequently analysed for whole-cell cAMP concentrations by radioimmunoassay. F(ab')_2 fragments of the monoclonal antibody 14-4.4S (anti-I-E), stimulated a rapid rise in cellular levels of cAMP (Fig. 2a). Similar results were obtained with other antibodies against Ia antigens including 10-2.16 (anti-I-A), but not with monoclonal antibodies against immunoglobulin (data not shown). These results suggest that the inhibitory effects of anti-Ia on the biological response of B cells may be mediated by an effect on cAMP levels in B cells.

If antibodies against Ia mediate their effects on PKC localization through cAMP, db-cAMP (dibutyl-cAMP) should also induce nuclear translocation of PKC. To test this possibility, small B cells were stimulated for 0–16 min with db-cAMP and fractionated as described in Fig. 1. Analysis of PKC activity in the resulting fractions revealed that db-cAMP induced transient nuclear translocation of PKC similar in kinetics and magnitude to the response induced by anti-Ia antibodies (Fig. 2b). Similar effects have been observed following stimulation of small B cells with 8-br-cAMP (8-bromo-cAMP), forskolin, cholera toxin and PGE_2 (data not shown).

To assess the ultrastructural localization of PKC associated with the nuclear fraction following stimulation with antibody against Ia or with db-cAMP, we conducted immunohistochemical analyses of PKC in nuclei from control or stimulated cells. Small B cells were stimulated, and nuclei were prepared as described above and stained by exposure to sheep antiserum directed against rat brain PKC^{12,21} or preimmune sera, followed by fluoresceinated rabbit anti-sheep immunoglobulin. After stimulation of cells for 2 min with monoclonal anti-I-A antibodies or 1 mM db-cAMP plus 5 mM theophylline, fluorescence indicative of PKC was found in apparent association with the nuclear envelope (Fig. 3a–d). This distribution is reminiscent of the distribution of histone proteins as determined by immunofluorescence^{22,23}.

Nuclei stained with antibody against PKC were also analysed by flow microfluorimetry to determine the proportion of cells which underwent the translocation response to db-cAMP and the magnitude of the increase in nucleus-associated PKC antigen. Figure 3e shows fluorescence histograms of anti-PKC

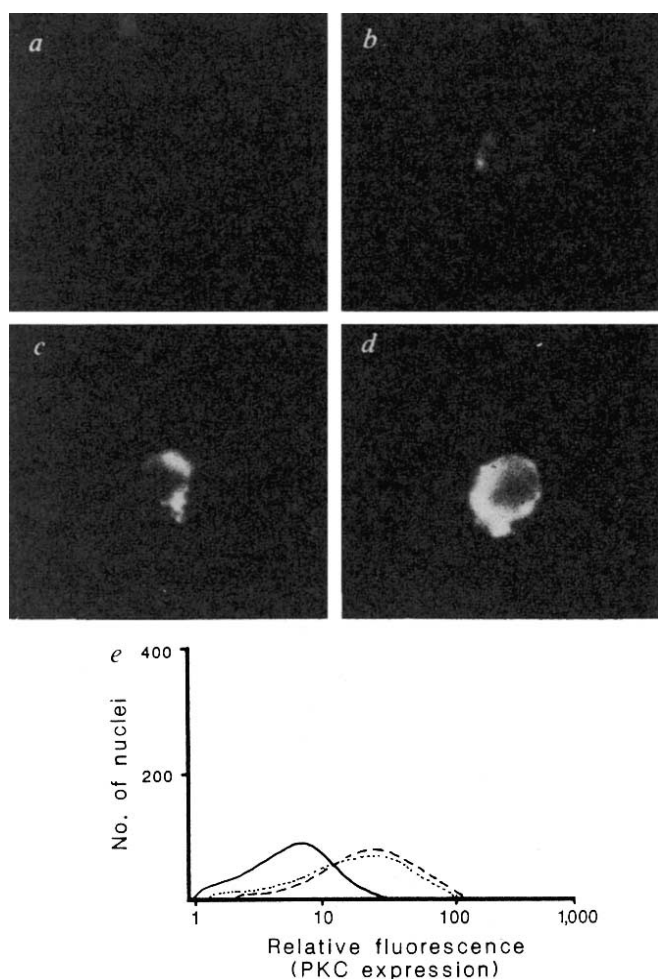


Fig. 3 Immunohistochemical localization of PKC in nuclei of db-cAMP or anti-Ia stimulated B cells. Quiescent B cells (AKR) were isolated as previously described¹⁰ and cultured at 10^7 ml^{-1} with no stimulus or in the presence of monoclonal anti-Ia antibodies, anti I-A (10.2.16) plus anti-I-E (14.4.4S) ($10 \mu\text{g ml}^{-1}$ each) or 1 mM db-cAMP plus 0.1 mM theophylline. After 2 or 4 min incubation, cells were cooled rapidly and nuclei were isolated as described in Fig. 1, and fixed using 3% formaldehyde. *b-d*, Fluorescence micrographs of single nuclei (magnification $\times 1,700$) from cells cultured with no stimulus (*b*), anti-Ia (*c*) or db-cAMP (*d*), which were stained with sheep anti-PKC² in conjunction with fluoresceinated rabbit anti-sheep IgG (Jackson ImmunoResearch). *a*, Nuclei from cells stimulated for 2 min with db-cAMP, which were stained with pre-immune serum and fluoresceinated rabbit anti-sheep IgG. Fluorescence histograms (three-decade log) of the anti-PKC-stained nuclei from unstimulated cells (solid line) or from cells stimulated for 2 min (broken line) or 4 min (dotted line) with 1 mM db-cAMP plus 0.1 mM theophylline. Histograms were generated by flow microfluorimetric analysis using an Ortho Cytofluorograf 50H with 2150 data handler. Nuclei were gated for analysis by forward narrow angle light scatter. Each histogram was based on analysis of 10,000 nuclei. Data are representative of three replicate experiments.

stained nuclei from unstimulated cells and cells stimulated for 2 min or 4 min with 1 mM db-cAMP plus 5 mM theophylline. Results indicate that most B cells respond to db-cAMP within 2 min by increasing nuclear PKC levels by approximately sixfold.

Finally we conducted Western blot analysis²⁴ to determine the molecular-weight characteristics of translocated PKC. Cytosol and nuclear fraction were prepared from control or db-cAMP-stimulated cells solubilized in SDS sample buffer, subjected to 10% SDS-PAGE and blotted using the sheep anti-PKC used above and peroxidase conjugated rabbit anti-sheep

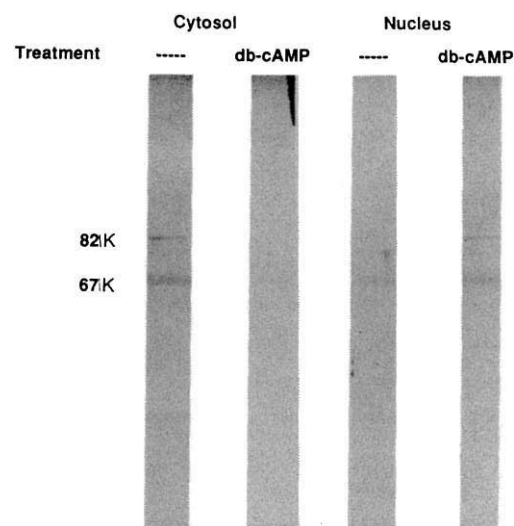


Fig. 4 Western blot analysis of db-cAMP induced translocation of PKC. Small B cells were cultured without stimulus (—) or stimulated with 1 mM db-cAMP plus 0.1 mM theophylline for 3 min before extraction with 0.5% NP40 as in Fig. 1. Nuclear and detergent-soluble fractions were separated by centrifugation (750g) through a 10% sucrose cushion. Nuclei were resuspended in SDS-sample buffer²⁴, disrupted by brief sonication and analysed along with the detergent soluble fraction by Western blot analysis²⁴ using 10% SDS-PAGE. Sheep anti-PKC²¹ and peroxidase-conjugated rabbit-anti-sheep IgG were used to localize PKC antigen. Cellular protein was used at $30 \mu\text{g}$ per lane. Results are representative of four replicate experiments. Sizes given are M_r .

immunoglobulin G (IgG). Predominant PKC species of relative molecular mass (M_r) 82,000 and 67,000 (82K and 67K) were seen in cytosol of unstimulated cells as previously reported²⁵ (Fig. 4). Within three minutes of stimulation these two antigenic activities disappeared almost totally from the cytosol. Concomitantly, the activity of the 82K and 67K species detectable in association with the nuclear fraction increased substantially. These findings indicate that both the 82K and the 67K PKC species are translocated to the nucleus following treatment of cells with db-cAMP.

These studies provide evidence that in B lymphocytes db-cAMP, or ligands which stimulate an increase in intracellular cAMP levels, stimulate the translocation of protein kinase C to a compartment associated with the nucleus. The distribution of this PKC appears similar to that of histone^{22,23} suggesting that this PKC may function in altering gene expression. This is supported by recent findings that histone H1, H2B and H4, and other nuclear proteins can be *in vivo* substrates for PKC²⁶⁻²⁹. It is further supported by preliminary findings which indicate that nuclear PKC can be eluted by conditions of high salt which elute histone from nuclei (Z.Z.C., unpublished observation). Although the specificity and molecular basis of PKC association with this nuclear material is unknown, it seems likely that it is mediated by protein kinase A (PKA) phosphorylation of PKC or a nuclear acceptor site. The latter possibility is consistent with previous reports that cAMP mediates the translocation of PKA to the nucleus³⁰⁻³¹. These possibilities are currently under study.

In contrast to anti-Ia effects, mIg crosslinking ligands activate phosphoinositide metabolism, Ca^{2+} mobilization and PKC translocation to the membrane, but not the nucleus. Therefore, in B cells, distinct translocative effects are mediated through diglyceride and Ca^{2+} , and cAMP. It is interesting that in B cells activation of these cascades has qualitatively distinct effects on cell function, the PtdIns metabolism cascade being associated with proliferation^{5,32}, and the cAMP cascade being associated

with differentiation^{11,18-20}. These findings are consistent with the possibility that the targets of these cascades are distinct gene subsets.

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Cloning and sequencing of human cholesteryl ester transfer protein cDNA

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The transfer of insoluble cholesteryl esters among lipoprotein particles is a vital step in normal cholesterol homeostasis and may be involved in the development of atherosclerosis¹. Extrahepatic tissues lack the enzymes required for the degradation of sterols to the excretable form of bile acids. Cholesterol synthesized in these tissues in excess of that needed for the synthesis of cell membranes or steroid hormones must accordingly be returned through the plasma to the liver for catabolism. The series of reactions involved has been termed reverse cholesterol transport¹. Catalysed steps of this pathway are believed to include an efflux from peripheral cells, which generates a diffusion gradient between these membranes and extracellular fluid; esterification of this cholesterol by lecithin-cholesterol acyltransferase (LCAT) (phosphatidylcholine-sterol acyltransferase) acting on species of high-density lipoproteins; transfer of the cholesteryl esters formed (largely to low- and very low-density lipoproteins) (LDL and VLDL) by a cholesteryl ester transfer protein (CETP); and removal of these lipoproteins, together with their cholesteryl ester content, by the liver through receptor-mediated and nonspecific endocytosis. Of these steps, the CETP reaction is the least characterized. Several laboratories have reported the purification from human plasma of proteins active on cholesteryl ester transfer between lipoprotein particles and possibly between cells and plasma²⁻⁵. However, the reported relative molecular mass (M_r), abundance and specificity of the purified activities have differed considerably. We have recently described the preparation of a highly active CETP of M_r 74,000 purified about 100,000-fold from human plasma, which may represent the functional component of earlier preparations⁶. Using a partial amino-acid sequence from this purified protein, CETP complementary DNA derived from human liver DNA has been cloned and sequenced and the cloned DNA used to detect CETP messenger RNA in a number of human tissues.

Amino-terminal sequence analysis of purified CETP yielded a provisional sequence for the first 24 residues⁷. Based upon that sequence data, probes of 39 and 72 nucleotides were synthesized representing a single codon choice for either amino-acid residues 10-22 or 1-24, respectively. About one million phage from an oligo (dT)-primed human adult liver cDNA library in λ gt10 were screened with each of the oligonucleotide probes⁷. Hybridizing plaques were purified and the cDNA inserts were subcloned into M13 vectors for sequence analysis⁸. Two apparent sibling clones detected with the 39-base probe were identified as bona fide CETP clones on the basis of agreement with protein sequence. The two clones, designated λ CETP.10 and λ CETP.11, contained cDNA inserts of 1,581 base pairs (bp). The translated cDNA sequence matched all but one of the amino acids from residues 10-24 of the protein sequence. The cDNA clones extended from the codon for residue 9 of the protein to the poly(A) tail at the 3' end of the message. No longer CETP clones were obtained in the initial screens. To recover the remainder of the CETP cDNA, libraries were rescreened with a 1,200-bp restriction fragment of λ CETP.10. Extensive screening of several liver cDNA libraries produced one clone, λ CETP.307, which extended farther in the 5' direction than λ CETP.10. This clone, with a cDNA insert of 1,100 bp, was recovered from a library in which reverse transcription was primed with random oligonucleotides rather than oligo(dT). The overlapping cDNA sequence of λ CETP.10 and λ CETP.307 is shown in Fig. 1. The DNA encodes part of a secretion signal prepeptide and a 476-amino-acid mature CETP protein. The predicted amino terminus agrees in 22 of 24 residues with direct protein sequence data we obtained from CETP. In addition, the cDNA sequence agrees with 13 of 17 residues of protein sequence we obtained from purified human lipid transfer protein-I (LTP-I) (John Albers, University of Washington⁴). (Because all amino-acid sequencing was performed with less than 50 pmol and with no chemical modification of cysteines, some residues were undetermined or assigned on a provisional basis. Therefore, the translated DNA sequence yields a more accurate determination of CETP sequence.)

After screening of $\sim 10^7$ plaques failed to yield a cDNA clone extending sufficiently far in the 5' direction to code for the complete signal prepeptide, a human genomic library in λ EMBL3 (ref. 9) was screened with clone λ CETP.10. Hybridizing plaques were rescreened with a 42-base oligonucleotide representing the 5' region of λ CETP.307, yielding the genomic clone designated λ CG.7. Oligonucleotide probing of restriction fragments of this genomic clone identified a 1,100-bp *Bgl*III fragment containing the 950 bp of 5' flanking and untranslated sequence, a methionine codon initiating a presumed 17-residue prepeptide and the initial codons of mature CETP. The genomic

Northern blot hybridization data (Fig. 2) show that liver is not the only site of synthesis of CETP mRNA. Human liver, small intestine and adrenal gland poly(A)⁺ RNA yield roughly comparable amounts of a 1,900-nucleotide species that hybrid-

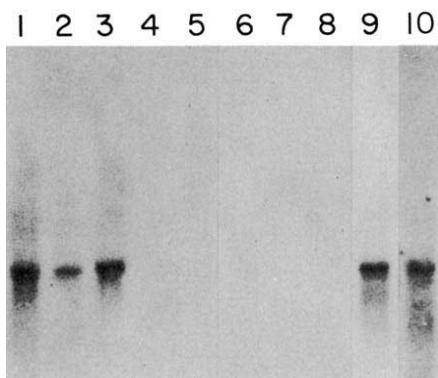


Fig. 2 Tissue distribution of CETP mRNA. Human poly(A)⁺ mRNA samples (15 µg) were electrophoresed in a 1.5% agarose, 6% formaldehyde gel; transferred to nitrocellulose²⁵, and hybridized with ³²P-labelled λCETP.10 cDNA using the conditions described in Fig. 1. RNA sources are: lane 1, liver; lane 2, small intestine (terminal ileum); lane 3, adrenal gland; lane 4, HepG2 (hepatocarcinoma) cells; lane 5, U937 (monocyte-like) cells; lane 6, pancreas; lane 7, white blood cells; lane 8, placenta; lane 9, spleen; lane 10, separated hepatocyte cells¹⁶. The CETP hybridizing band measures 1,900 ± 50 nucleotides. All lanes are from the same experiment, but lanes 1–8 were exposed to X-ray film for 24 h, and lane 9 for 5 h.

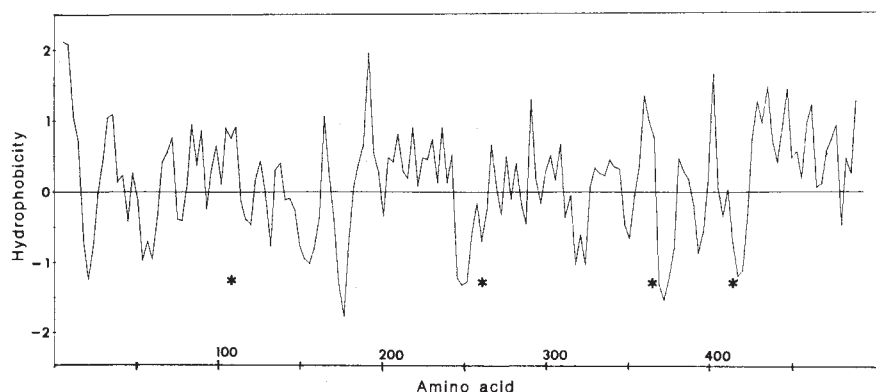


Fig. 3 Hydropathy profile of CETP. The translated DNA sequence of human CETP was plotted using the algorithm of Kyte and Doolittle¹². The abscissa denotes amino acids beginning at the signal prepeptide. Positive points in the ordinate denote hydrophobic regions of the protein; each point represents the average hydropathy of ten successive amino acids in the sequence. Asterisks, possible asparagine-linked glycosylation sites. The overall index of hydropathy = 0.10, which is more hydrophobic than LCAT and the apolipoproteins that have been sequenced to date.

izes with the cloned CETP cDNA. Spleen RNA has a greater amount of CETP message, whereas little or no hybridization is seen with RNA prepared from HepG2 cells, U937 cells, pancreas, white blood cells or placenta. In addition, no hybridizing RNA could be detected in lung, kidney or fetal brain (data not shown). The CETP mRNA in spleen may arise from activated lymphocytes or from macrophages, which have been shown to secrete a lipid transfer protein¹⁵. It was also suggested¹⁵ that because ~10% of the total liver mass may be composed of macrophage-like cells, it might be these cells which account for all of the hepatic lipid transfer activity. However, CETP mRNA was detected in isolated hepatocytes¹⁶ (lane 10), and the synthesis of CETP has now been demonstrated in the hepatocarcinoma cell line HepG2 (ref. 17). (The fact that there is no hybridizing mRNA in lane 4 may be due to detection limits or to the procedures of cell growth.) In attempting to interpret the Northern blot results, we note that several protein components of lipoprotein particles, such as LCAT⁷ and apolipoproteins A-I, A-II, A-IV, B and C-III are synthesized primarily in liver or in liver and intestine^{18–20}. These are the tissues that are most active in the secretion of intact lipoproteins. In contrast apolipoproteins D and E are synthesized in a wide variety of tissues, with the concentration of mRNA in adrenal tissue being close to, or greater than that in liver^{21–23}. Apolipoprotein synthesis in peripheral tissues might assist the removal of cholesterol from certain cells, or the delivery to them of cholesterol for cell membrane biosynthesis or for the production of steroid hormones. Just as the exchange of free cholesterol between cells and plasma might be facilitated by apolipoproteins that were first characterized as components of circulating lipoproteins, CETP may take part in the exchange of cholesteryl esters between cells and plasma, in addition to its function in the exchange of core lipids between circulating lipoprotein particles²⁴. This might be of particular importance in the unloading of macrophages containing free and esterified cholesterol, such as make up the foam cells found in atherosclerotic lesions. Future experiments with hybridization of CETP probes to RNA

extracted from tissues and *in situ*, and with the transfer protein purified from cell cultures expressing the cloned gene, should help to elucidate activities of CETP in normal and in pathological cholesterol homeostasis.

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Note added in proof: As further confirmation of identity, transfection of cultured mammalian cells with cloned CETP DNA leads to secretion of cholesteryl ester transfer activity.

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A novel calcium binding site in the galactose-binding protein of bacterial transport and chemotaxis

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The refined 1.9-Å resolution structure of the periplasmic D-galactose-binding protein (GBP) reveals a calcium ion surrounded by seven ligands, all protein oxygen atoms. A nine-residue loop (amino-acid positions 134–142), which is preceded by a β -turn and followed by a β -strand, provides five ligands from every second residue. The last two ligands are supplied by the carboxylate group of Glu 205. The entire GBP Ca^{2+} -binding site adopts a conformation very similar to the site in the 'helix-loop-helix' or 'EF-hand' unit commonly found in intracellular calcium-binding proteins¹, but without the two helices. Structural analyses have also uncovered the sugar-binding site some 30 Å from the calcium and a site for interacting with the membrane-bound *trg* chemotactic signal transducer ~45 Å from the calcium. Our results show that a common tight calcium binding site of ancient origin can be tethered to different secondary structures. They also provide the first demonstration of a metal-binding site in a protein which is involved in bacterial active transport and chemotaxis.

GBP is the first of a large group of proteins that participate in both active transport and the simple behavioural response of bacterial chemotaxis to be discovered^{2,3}. These proteins consist of single polypeptide chains with similar tight substrate binding sites ($K_d \approx 0.1 \mu\text{M}$). Besides GBP, the structure and function of six other binding proteins (of relative molecular masses (M_r) 33,500–40,500) with specificities for L-arabinose, sulphate, leucine/isoleucine/valine, leucine, D-maltose and phosphate are currently under investigation in our laboratory, principally by X-ray crystallography (refs 4–8, and unpublished data). These proteins are ellipsoid and are composed of two similar globular domains. They contain a deep cleft between the two domains

where substrates are bound primarily by hydrogen bonds and become almost completely engulfed^{4–5,7,9}.

Crystallographic refinement of the structure of GBP with bound glucose, also a natural substrate, at 1.9-Å resolution proceeded to an *R*-factor of 0.146 for 19,531 measured reflections (no σ cut-off applied) in the shell from 10 to 1.9 Å (Fig. 1 legend for additional refinement statistics). The GBP structure is one of the better refined calcium-binding protein structures. The entire structure, which consists of 309 residues, is well defined, especially in the regions of the substrate- and metal-binding sites (Fig. 1). The structure determination and the mode of binding of the sugar will be reported in detail elsewhere.

As metallic cation was not added to the crystallizing medium and was not previously seen in refined structures of the arabinose- and sulphate-binding proteins (which are essential in transport but not in chemotaxis)^{4,5}, its existence in GBP was a complete surprise. The bound metal was discovered in the course of careful and exhaustive analysis of bound water molecules, near the conclusion of the structure refinement. All evidence points to the identification of a metal binding site with specificity, later established by further refinement and independent metal analysis, for calcium.

Figure 1*b* shows the atomic structure around the Ca^{2+} which is coordinated by seven ligands, all protein oxygen atoms. A peptide segment consisting of residues 134–142 loops around the calcium and supplies ligand atoms Asp 134 OD1, Asn 136 OD1, Asp 138 OD1, Gln 140 O, and Gln 142 OE1. The remaining two ligands are provided by Glu 205 OE1 and OE2 atoms. The Ca^{2+} coordination is an almost pentagonal bipyramid, with apices occupied by oxygen atoms from the side chains of the first and ninth positions of the loop. The average Ca^{2+} –O distance is 2.41 (0.10) Å which is in excellent agreement with distances observed in crystal structures of small calcium complexes. As the calcium binding loop looks like a lock-washer, with Glu 205 seemingly keeping both ends of the loop flared through hydrogen-bonding (Fig. 1*b*), we will refer to the entire structure as the 'lock-washer'.

The Ca^{2+} -binding site is in the C-terminal domain of the protein, at one end of the ellipsoidal, two-domain structure (Fig. 2). The nine-residue loop, the main constituent of the metal site, is immediately preceded by a reverse turn (type I) and followed by the first strand in the C-terminal domain. The reverse turn itself is preceded by the first α -helix in the domain. Residues

Table 1 Sequence alignment of Ca^{2+} -binding sites in GBP and EF-hand loops

	1	2	3	4	5	6	7	8	9	10	11	12	
	*		*		*		*		*			*	
GBP													
Site	134	Asp	Leu	Asn	Lys	Asp	Gly	Gln	Ile	142		204	
Parvalbumin										Gln	—	Ile	Glu
Loop CD	51	Asp	Gln	Asp	Lys	Ser	Gly	Phe	Ile	Glu	Glu	Asp	Glu
Loop EF	90	Asp	Ser	Asp	Gly	Asp	Gly	Lys	Ile	Gly	Val	Asp	Glu
Troponin C													
Loop I	30	Asp	Ala	Asp	Gly	Gly	Gly	Asp	Ile	Ser	Thr	Lys	Glu
Loop II	66	Asp	Glu	Asp	Gly	Ser	Gly	Thr	Ile	Asp	Phe	Glu	Glu
Loop III	106	Asp	Lys	Asn	Ala	Asp	Gly	Phe	Ile	Asp	Ile	Glu	Glu
Loop IV	142	Asp	Lys	Asn	Asn	Asp	Gly	Arg	Ile	Asp	Phe	Asp	Glu
ICaBP													
Loop III-IV	54	Asp	Lys	Asn	Gly	Asp	Gly	Glu	Val	Ser	Phe	Glu	Glu
Calmodulin													
Loop I	20	Asp	Lys	Asp	Gly	Asn	Gly	Thr	Ile	Thr	Thr	Lys	Glu
Loop II	56	Asp	Ala	Asp	Gly	Asn	Gly	Thr	Ile	Asp	Phe	Pro	Glu
Loop III	93	Asp	Lys	Asp	Gly	Asn	Gly	Tyr	Ile	Ser	Ala	Ala	Glu
Loop IV	129	Asn	Ile	Asp	Gly	Asp	Gly	Glu	Val	Asn	Tyr	Glu	Glu

The sequence of the calcium binding site in GBP (galactose-binding protein)¹⁷ is compared with EF loops of (parvalbumin)¹⁸, troponin C¹⁹, ICaBP (vitamin-D-dependent intestinal calcium binding protein)²⁰ and calmodulin²¹. Calcium ligands, determined crystallographically or predicted on the basis of sequence homology, are marked by asterisks. The ligand at position 7 is provided by the peptide carbonyl oxygen while the others are from side chain oxygens.

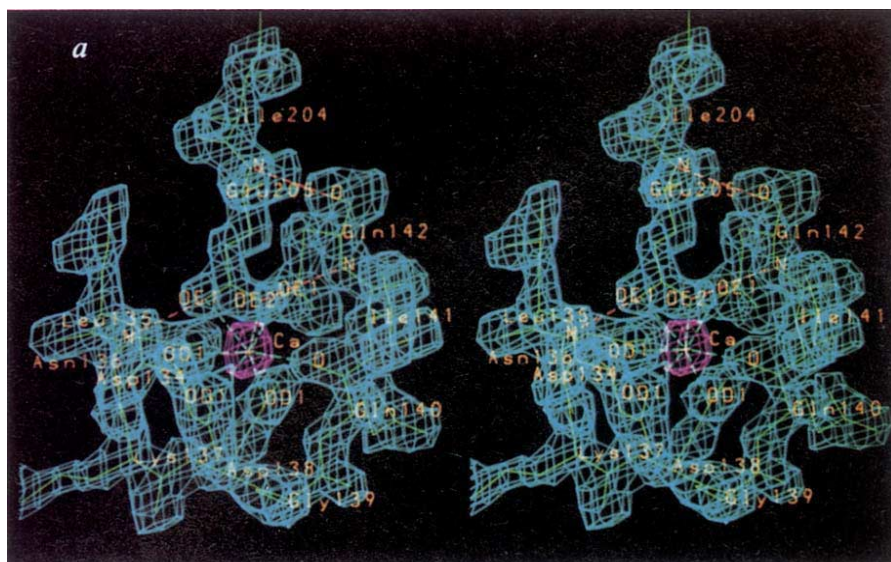


Fig. 1 *a*, Stereoscopic view of the atomic structure of the D-galactose-binding protein (light yellow) in the immediate vicinity of the bound calcium superimposed on the electron density map (blue). The calcium density is distinguished in the figure by a magenta colour. The electron density was calculated with the use of coefficients $[(2|F_{\text{obs}}| - |F_{\text{calc}}|), \alpha_{\text{calc}}]$ and contoured at one σ of the electron density map. Coefficients were obtained from restrained-parameter least-squares refinement²² at 1.9-Å resolution. The entire structure consists of a total of 2,576 non-hydrogen atoms, comprising 2,348 from the entire sequence of 309 amino acid residues, 12 from one sugar anomer, 1 Ca^{2+} and 215 water molecules. The root-mean-squares deviations from accepted protein stereochemistry are: 0.024 Å for bond distances, 0.045 Å for bond angle distances, 0.012 Å for deviations from planarity of planar groups, 6.6° for deviation from 180° for the ω angle of the peptide groups, and 0.10 Å³ for the chiral volume. The PS300/FRODO graphics program²³ was used in the structure analysis. *b*, Identical to *a* but with the density surface removed to show more clearly the calcium-binding site (yellow structure) and the metal coordination (broken green lines). The calcium is shown with its metal surface radius as dots. The calcium-binding site is composed of a loop containing residues 134–142 and a dipeptide segment consisting of residues 204–205. The H bonds involving Glu 205 are represented by red dashed lines; other H bonds (not shown) which stabilize the conformation of the loop are discussed in the text.

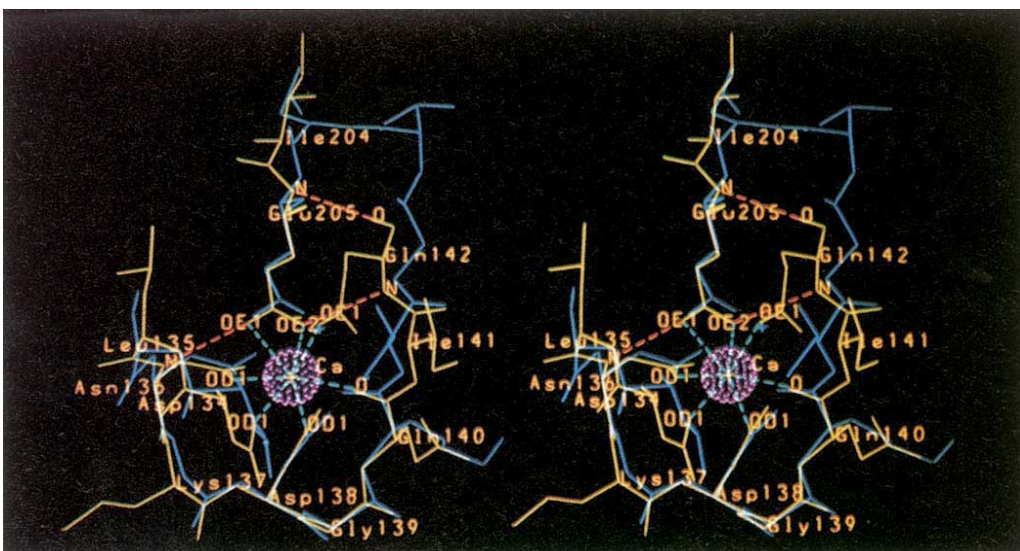
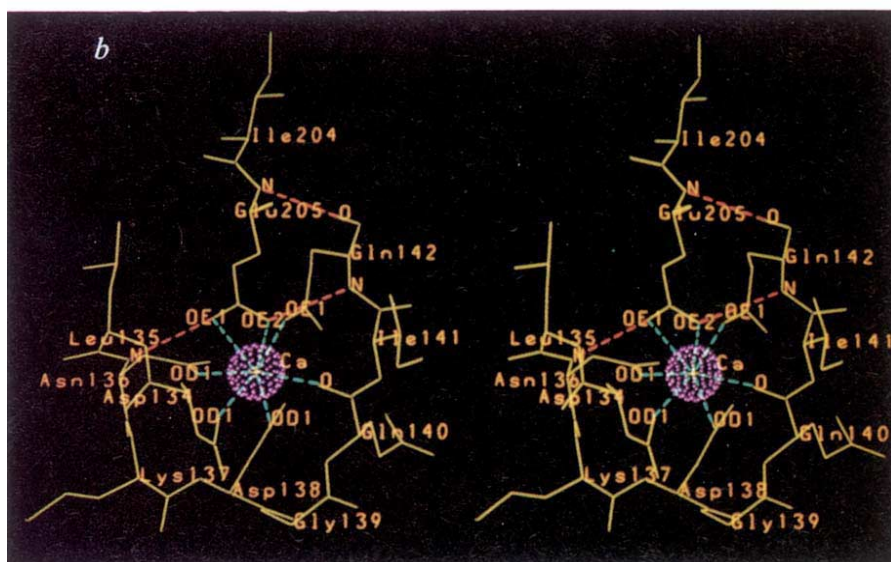
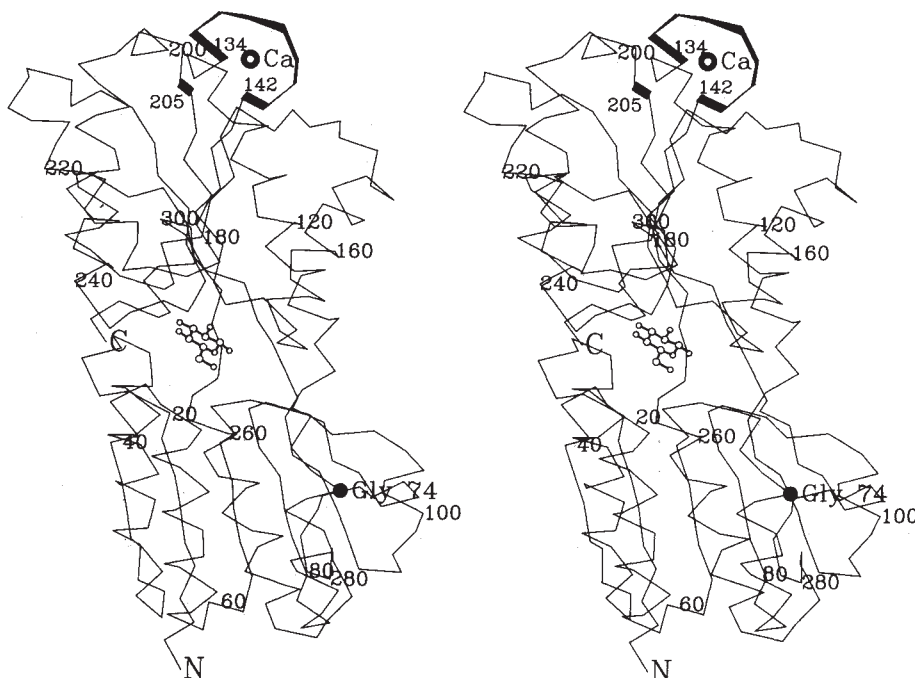


Fig. 3 Comparison of the conformation of the calcium-binding site in GBP (yellow) and the site in the loop of parvalbumin (blue). The 44 main-chain atoms of residues 134–142 and 204–205 that make up the Ca^{2+} -binding site in GBP and the calcium are optimally superimposed with corresponding atoms of the site in the EF loop (data A6 in the protein data bank²⁴), resulting in an r.m.s. deviation of 0.60 Å. Blue asterisk, a water ligand in the EF loop. Amino acid sequences are listed in Table 1 for comparison.

Fig. 2 C_{α} backbone trace of GBP showing the locations of the binding sites for calcium, β -D-glucose, and the *trg* signal transducer. N, amino-terminal end; C, carboxyl end. Numbers are given for every twenty residues and for residues in the metal and transducer sites. The GBP structure is ellipsoid (axial ratio $\approx 2:1$) and is composed of two very similar globular domains which are designated N- and C-domains as the former contains the N-terminus and the latter the C-terminus. The calcium (Ca, thick circle) is surrounded by residues highlighted by thicker lines between C_{α} atoms. The 9-residue metal binding loop (134–142) is immediately preceded by a type-I reverse turn (131–134) and followed by β -strand (142–147). The reverse turn is preceded by the first helix (113–130) in the C-domain. The dipeptide 204–205, also a part of the metal site, is located near the beginning of the third strand in the domain. Gly 74 (filled circle) is part of a site for interacting with the membrane-bound *trg* signal transducer. The sugar, which is at the centre of GBP, is 30 Å from the bound calcium and 18 Å from the *trg* site.



Leu 204 (also structurally a part of the metal site) to Glu 205 lie near the beginning of the third β -strand of the C-domain's.

In addition to the periplasmic binding proteins, distinct protein components in the cytoplasmic membrane are required for either active transport or chemotaxis^{10–11}. These components somehow interact with the liganded form of the binding proteins, initiating the translocation process or flagella motion. To locate and characterize a site in GBP for interaction with the membrane-bound *trg* signal transducer, we have also solved and refined the X-ray structure of a mutant GBP isolated from AW551 strain of *Escherichia coli*¹² which is defective in chemotaxis toward galactose, but fully competent in sugar transport, and compared it with the wild-type structure (Vyas *et al.*, manuscript in preparation). The transducer attachment site, which includes Gly 74, is 45 Å from the calcium binding site (Fig. 2). The sequence analysis which shows that Gly 74 is substituted by an aspartyl residue in the mutant protein¹³ is confirmed by the structural studies. The metal in both native and mutant GBP is 30 Å from the sugar substrate bound in the cleft formed between the two domains (Fig. 2).

The metal binding site in GBP is unique. Helices are not directly linked to the site; nevertheless, we find great structural and sequence similarities with the sites in the class of calcium-binding folds, better known as the EF-hand, commonly found in calcium-modulated intracellular proteins^{1,14,15} (Fig. 3 and Table 1). In particular, the nine-residue loop in GBP adopts a conformation that is very similar to the first 9 residues of the 12-residue EF loop. Moreover, Glu 205, which deploys bidentate ligands to the metal, occupies a position equivalent to the invariant residue at coordinating position 12 of the EF loop, and the backbone atoms of residue 204 partly superimpose with those of the residue at position 11. Thus only position 10 of the EF loop is without any structural equivalence in the lock-washer, but this position and position 11 as well contain variable side chains (Table 1), none of which appear to be critical in binding the metal or in maintaining structural integrity of the EF loop. It is important to note that the root-mean-squares (r.m.s.) discrepancy of 0.60 Å between main-chain coordinates of the GBP

Ca^{2+} -binding site superimposed on the parvalbumin EF loop (Fig. 3) compares very well with values obtained from comparisons of several EF hand proteins¹⁵.

There are other notable similarities between the lock-washer and EF loop. The nine-residue loop in the lock-washer contains residues very similar to the highly conserved ones of many EF-hand loops (Table 1 and Fig. 3). These key residues include those that contain side-chain ligands at positions 1, 3 and 5 and those that confer structural constraint or stability located at positions 6 and 8. Moreover, in both the lock-washer and EF loops, the coordinating atom at position 7 is provided by the peptide carbonyl oxygen. In this conjunction, Gly 139 at position 6 is crucial as it, unlike residues with side chains, adopts a main-chain conformation ($\phi = 70^\circ$, $\psi = 5^\circ$) which ensures that the chain direction changes appropriately to allow the peptide carbonyl oxygen of the residue in position 7 to coordinate the calcium. The glycyl conformation is stabilized by a hydrogen bond between Gly 139 peptide NH and Asp 134 OD2. On the other hand, the branched aliphatic side chain at position 8 is directed toward the interior of the domain, thereby contributing to stability of the loop. We have also noted previously the invariant glutamyl residue at position 12 (Table 1).

Lastly, both the conformations of the EF loop and the lock-washer are stabilized by hydrogen bonds. In EF-hand proteins these hydrogen bonds are in the forms of reverse turns (one at the start and another at the end of the loop), several 'Asx turns' and a β -sheet¹⁵. A total of nine excellent hydrogen bonds are formed in the lock-washer: three between main-chain atoms only, four between main-chain and side-chain atoms, and two between side chains. There is a reverse turn located at the start of the nine-residue loop, which is followed by an Asx turn. A parallel β -sheet hydrogen-bonding occurs between the residue 141 (position 8 residue) and residues 172 and 174 of an adjacent strand (Fig. 2). The other H-bonds do not fall under any categories.

Not only is the lock-washer homologous to the EF loops, but it shares a common feature with the CD-hand loop of parvalbumin¹ by also having no water molecule as ligand to the

calcium. This common feature is based on the fact that the side chain of the residue at position 9 (Gln in GBP and Glu in the CD loop, Table 1) is directly liganded to the calcium ion. In contrast it has been shown that if the side chain at position 9 is too small, a common occurrence among the EF loops (Table 1), then a water molecule is found intervening between the side chain and the calcium¹⁵.

The remarkable results presented above suggest a variety of calcium binding folds or units, all containing very similar cation binding sites. Because the binding sites of these units can be tethered to different types of secondary structure (helices, β -strands, reverse turns), the conformational integrity of the metal site depends almost entirely on the hydrogen bonds, non-polar interactions and other structural features characteristic of the site itself (see above). To achieve the requisite conformation the reverse turns at the start and the end of the site are of considerable importance. These turns could be part of the carboxyl- and amino-termini of helices, as in the case of the EF loops, or could be separate entities as found at the beginning of the lock-washer loop. Interestingly, although the GBP calcium site is composed of two separate peptide segments, the main chain CO group of the residue at position 9 (Gln 142) is hydrogen-bonded to the peptide NH group of the residue at position 12 (Glu 205), thus mimicking a reverse turn. Not only is it possible to have different types of secondary structure in the metal binding units, but, as recently shown in the case of the helix-loop-helix unit (or 'elbow') in α -lactalbumin¹⁶, the calcium-binding loop itself need not closely conform to the EF loop. Note that the metal binding site in GBP appears to be as stable as the site in the helix-loop-helix; the B -values of about 15 Å² for the ion and its ligands in GBP are very similar to those of the highly refined lactalbumin structure¹⁶.

Although the bacterial galactose-binding protein has no evolutionary relationship with the eukaryotic EF-hand proteins, the results presented here indicate that the calcium binding site (especially the peptide loop) in all these proteins appears ancient and designed to provide extremely tight and specific binding of the biologically important cation.

What is the function of the calcium? At this stage of our studies, we cannot be certain of the structural and functional role of the bound calcium. Minimally, the calcium could confer stability to GBP. It could also have a function in excitatory signal. The discovery of calcium bound to a periplasmic binding protein has not only prompted a variety of studies, but drawn attention to the designs, chemistry, and evolution of calcium binding sites and to the ever-widening importance of the cation in biological processes.

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Preferential relaxation of supercoiled DNA containing a hexadecameric recognition sequence for topoisomerase I

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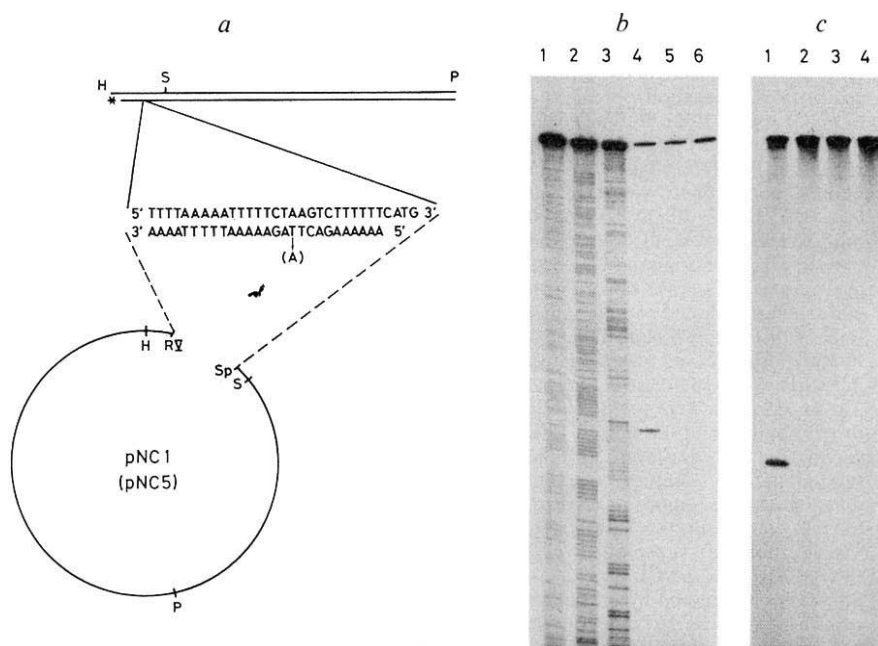
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In prokaryotes, the degree of supercoiling of DNA can profoundly influence the use of specific promoters (for review, see ref. 1). In eukaryotes, a variety of indirect observations suggest that DNA topology has a similar importance in proper gene expression²⁻⁴. Much attention has therefore been focused on the cellular proteins that control DNA supercoiling, among which are the enzymes topoisomerase I and II (for review see refs 5 and 6). A hexadecameric sequence functions as a strong attraction site for topoisomerase I⁷. Here we report that the interaction of topoisomerase I with this sequence motif is highly specific, because a single base-pair substitution prevents strand cleavage and thereby catalytic activity at the sequence. Thus, supercoiled DNA containing the recognition sequence is relaxed preferentially by topoisomerase I compared to a control, but no difference in the relaxation rate is observed for supercoiled DNA carrying the mutated sequence. The preference for the recognition sequence seems to be an intrinsic property of all eukaryotic type I topoisomerases⁸, suggesting that the interaction might be important in a fundamental biological process.

Topoisomerase I catalyses changes in the topological state of duplex DNA by concerted breakage and reclosure of one strand of the DNA. The temporary nick provides a swivel which allows the linking number to change, while a covalent linkage between the enzyme and the broken strand stores the energy required for the reclosure. Treatment of a topoisomerase I/DNA complex with strong protein denaturants results in single strand breakage and covalent linkage of topoisomerase I to the 3' end of the broken DNA^{5,6}. Formation of such covalent DNA/protein complexes may therefore be considered as an abortive reaction of the normal catalytic cycle: 'nicking' without 'closing'. Recently, accumulation of nicked topoisomerase I/DNA intermediates has been used to map binding sites for topoisomerase I in the eukaryotic genome⁷⁻⁹. However, it has been argued that the nicked intermediates represent artefacts accumulated at sequences where closing is rate-limited or blocked and thus do not represent true intermediates in the catalytic cycle. To investigate this phenomenon further and to determine the sequence specificity of topoisomerase I we have compared the formation of topoisomerase I/DNA covalent intermediates with the ability of the enzyme to relax supercoiled DNA containing specific sequences.

Topoisomerase I has a strong binding preference for the hexadecameric sequence motif AGACTTAGAGAAA^{TTT} (refs 7 and 8). This sequence motif flanks a number of eukaryotic genes, especially ribosomal RNA genes, where it is often located in DNaseI-hypersensitive sites⁷. To investigate the interaction between topoisomerase I and a single recognition sequence in a foreign DNA background, a 27-base pair (bp) oligonucleotide containing the recognition sequence AGACTTAGAGAAAATTT

Fig. 1 Recognition by topoisomerase I of a specific hexadecameric sequence. *a*, Schematic representation of the recombinant plasmids pNC1 and pNC5 used for the cleavage induction and relaxation in the presence of topoisomerase. A synthetic 27-bp oligonucleotide containing the topoisomerase I recognition sequence was inserted into the *EcoRV*-*SphI* sites of pBR322 giving rise to the plasmid pNC1. Plasmid pNC5 is identical to pNC1 except that the T in position 11 measured from the 5' end of the *SphI* site has been replaced by an A. Abbreviations: H, *HindIII*; Sp, *SphI*; P, *PvuII*; S, *Sall*. *b*, Cleavage induction was investigated on a gel-purified 276-bp *HindIII*-*Sall* fragment of pNC1, 3' end-labelled at the *HindIII* site as indicated in *a*. The labelled fragment (3 fmol) was incubated with 15 units of homogeneous *Tetrahymena* topoisomerase I for 15 min at 30 °C: lane 4, cleavage induction of the topoisomerase-DNA complexes formed, by addition of 1% SDS before 1 M NaCl; lane 5, dissociation of the topoisomerase-DNA complexes formed, by 1 M NaCl before the addition of 1% SDS; lane 6, addition of 1% SDS to the DNA fragment incubated in the absence of topoisomerase. The samples were thereafter treated with proteinase K, phenol extracted, alcohol precipitated and loaded on a sequencing gel and run in parallel to the fragment subjected to Maxam-Gilbert chemical degradation¹²: lane 1, A > C reaction; lane 2, G + A; lane 3, C > T. *c*, Cleavage induction was investigated on a gel purified 1.6-kb *HindIII*-*PvuII* fragment of pNC1 and pNC5, respectively. The fragments were labelled at the *HindIII* sites as in *b*. The labelled fragments (3 fmol) were incubated with 15 units of homogeneous *Tetrahymena* topoisomerase I for 15 min at 30 °C. Cleavage was induced by addition of 1% SDS to the topoisomerase-DNA complexes formed: lane 1, the fragment of pNC1; lane 2, the fragment of pNC5. Dissociation of the topoisomerase-DNA complexes by 1 M NaCl before addition of 1% SDS: lane 3, the fragment of pNC1; lane 4, the fragment of pNC5. In all experiments homogeneous topoisomerase I from *Tetrahymena* with a specific activity of 5×10^6 units per mg protein was used. One unit of enzyme equals the activity giving 50% relaxation of 1 μ g supercoiled pBR322 in 30 min at 30 °C.



was synthesized and inserted between the *EcoRV* and *SphI* sites of pBR322 (Fig. 1*a*). Binding of topoisomerase I to the sequence was monitored by the formation of cleavable complexes upon addition of SDS. Figure 1*b* shows the topoisomerase I-dependent cleavage on a 276-bp *HindIII*-*Sall* fragment, 3' end-labelled at the *HindIII* site (lane 4). Electrophoresis of the cleaved fragment in parallel to the 3' end-labelled 276-bp fragment subjected to Maxam-Gilbert chemical degradation allows the sequence at the cleavage site to be read (lanes 1-3). Only one cleavage site is observed in the fragment incubated with topoisomerase I, and sequence analysis demonstrates that it is confined to the inserted 27-bp sequence. Cleavage is between the T and A residues at positions 11 and 12, respectively, measured from the 5' end of the *SphI* site. Densitometry of the autoradiogram reveals that ~25% of the fragment is cleaved at the recognition sequence. Cleavage is abolished by dissociation of the enzyme-DNA complex with 1 M NaCl (lane 5) before addition of SDS, or by incubation of the fragment in the absence of topoisomerase I (lane 6). Substitution of a single base in the recognition sequence inhibits cleavage by more than 95% as is seen from Fig. 1*c*, lane 2, where the T in position 11 has been replaced by an A. Cleavage was in this case investigated on a 1.6-kilobase (kb) *HindIII*-*PvuII* fragment from the clone pNC 5 (Fig. 1*a*) containing the mutated sequence. Control experiments with an identical DNA fragment of the clone pNC1 (wild-type sequence) reveal 25% cleavage at the recognition sequence (lane 1). Cleavage occurs, as shown for the smaller *HindIII*-*Sall* fragment in Fig. 1*b* at the predicted site between nucleotides 11 and 12 in the inserted sequence, whereas the other part of the fragment remains uncleaved. Incubation of the fragment in the absence of topoisomerase I or dissociation of the enzyme-DNA complex with salt abolishes cleavage (lanes 3-4).

Because sites of topoisomerase cleavage can easily be mapped either in purified systems or *in vivo*, extensive studies have been

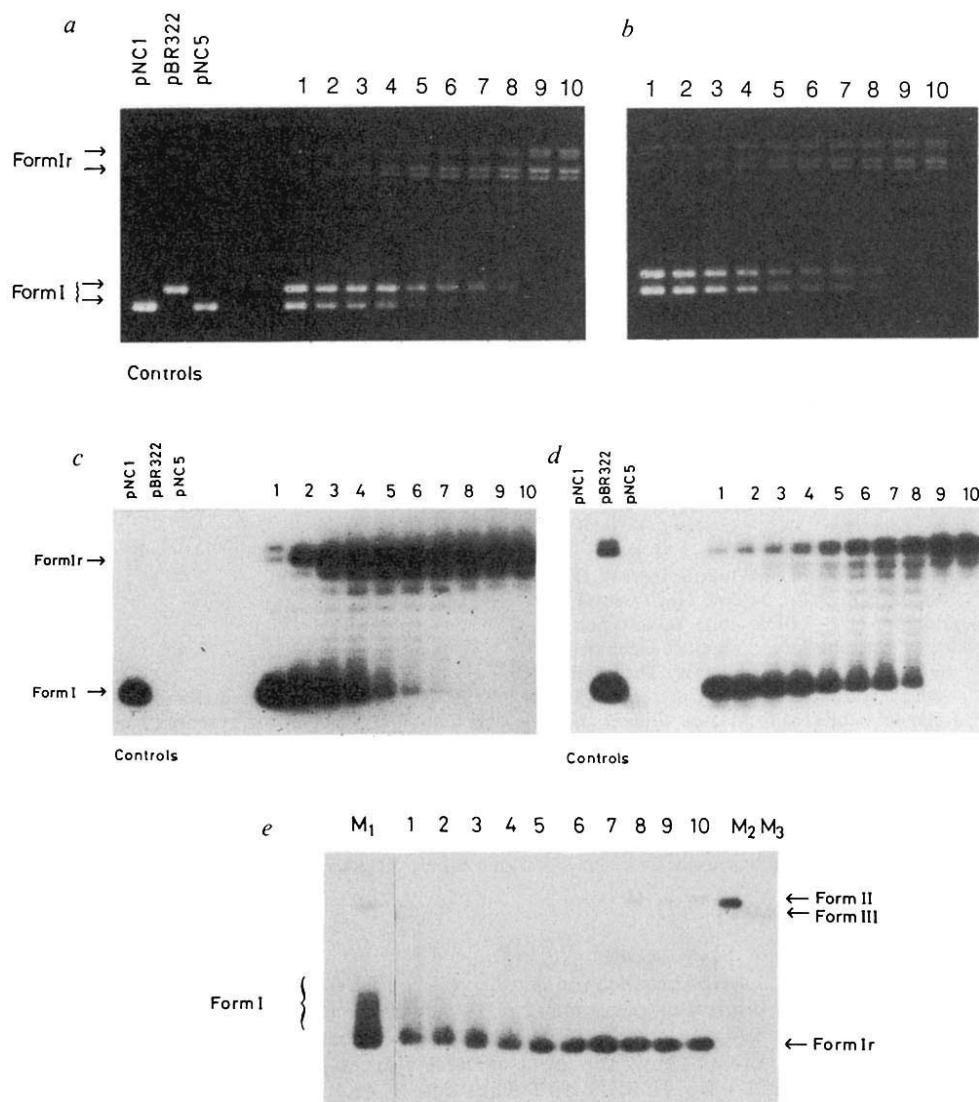
carried out on cleavage of DNA in the presence of topoisomerase. Strong DNA binding sites that show little cleavage upon addition of protein denaturants to the enzyme-DNA complex are known; thus strong binding sites for topoisomerase are not necessarily strong cleavage sites or preferential sites in catalysis of DNA breakage and rejoining^{1,10}. To investigate if the cleavage at the hexadecameric sequence is also a preferential catalytic site for topoisomerase I, relaxation kinetics were investigated in a mixture of the supercoiled plasmids pNC1 and pBR322 (Fig. 2*a*). We found preferential relaxation of the smaller plasmid pNC1, suggesting that topoisomerase I specifically interacts with the recognition sequence in this plasmid. To exclude alternative explanations, relaxation was studied in a mixture of pNC5 and pBR322 (Fig. 2*b*). In contrast to pNC1, supercoiled pNC5 is relaxed with the same rate as the control plasmid. This demonstrates that both at the level of cleavage induction and relaxation, topoisomerase I recognizes a single sequence element in the plasmid and that a single base-pair substitution in the element abolishes catalytic activity at the site. The mixtures pNC1-pBR322 and pNC5-pBR322 are both fully relaxed after longer times of incubation with topoisomerase I (Fig. 2*a* and *b*, lanes 9-10). Southern blotting of the gel shown in Fig. 2*a* and hybridization with ³²P-labelled probes specific for the plasmid pNC1 (Fig. 2*c*) or the plasmid pBR322 (Fig. 2*d*) revealed that the plasmid pNC1 containing the recognition sequence is relaxed ~10-fold faster than pBR322. Electrophoresis of the DNA samples used in Fig. 2*a* in the presence of chloroquine demonstrates that >95% of the molecules are present in a covalently closed form, excluding the accumulation of nicked intermediates (Fig. 2*e*).

The data presented demonstrate that topoisomerase I from *Tetrahymena* has a strong preference for a hexadecameric sequence element. Similar results have been obtained with

Fig. 2 Topoisomerase I relaxes preferentially supercoiled DNA containing a hexadecameric recognition sequence. *a*, Relaxation of a mixture of pNC1 and pBR322 (2 μ g of each) by homogenous topoisomerase I (3 units). The mixture was incubated at 30 °C and aliquots were taken at 0, 1, 3, 5, 10, 15, 20, 30, 60 and 90 min. The reaction was stopped, and the samples were analysed on a 1% agarose gel. The gel was stained with ethidium bromide and photographed under UV-light. *b*, Relaxation of a mixture of pNC5 and pBR322 (2 μ g of each) by topoisomerase I (3 units). The experiments were performed as in *a*. In both *a* and *b* a few positive supercoils are displayed in the relaxed DNA samples due to differences in conditions in reaction mixture and electrophoresis. *c*, Visualization of supercoiled and relaxed forms of pNC1 from *a* by Southern blotting and hybridization with the 32 P-labelled hexadecameric recognition sequence. *d*, Visualization of supercoiled and relaxed forms of pBR322 from *a* by Southern blotting and hybridization with a probe specific for the 377-bp long *EcoRV-SphI* fragment. *e*, Visualization of covalently closed and nicked DNA molecules in experiment *a*. The DNA samples were electrophoresed on a 1% agarose gel in the presence of saturating amounts of chloroquine, and visualized by Southern blotting followed by hybridization with the 32 P-labelled hexadecameric recognition sequence specific for pNC1. The markers M_1 , M_2 and M_3 are supercoiled (form I), relaxed (form Ir), nicked circular (form II) and linearized (form III) DNA molecules, respectively.

Methods. Relaxation studies were done at 30 °C in a buffer containing

10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.01 mM EDTA and 20 μ g ml $^{-1}$ of BSA. Agarose gel electrophoresis, Southern blot, hybridization and 32 P-labelling of the probes by 3' or 5' end labelling followed standard procedures⁷. Hybridization with the hexadecameric recognition sequence was done at 37 °C in the presence of 6 $\times$ SSC. Chloroquine gels were run as in ref. 13.



topoisomerase I from human cells (unpublished results). This preference is exerted both in cleavage and in catalysis of DNA breakage and rejoining, thereby establishing the equivalence between cleavage and relaxation sites. Based on the notion that in general topoisomerase I interaction sites occur for every 1–2 helical turns of the random sequence of duplex DNA¹¹, 200–400 sites are expected to appear in pBR322. The 10-fold faster relaxation rate of pNC1 relative to pBR322 thus indicates that the relaxation rate at the recognition sequence exceeds the rate at an average site by as much as 4,000-fold. Studies *in vivo* and *in situ* have revealed that topoisomerase I is tightly associated with identical recognition sequences on the chromatin^{7,8}. When these data are considered together with the observation that the binding preference for the recognition sequence is an intrinsic property of all eukaryotic type I topoisomerases^{7,8}, it seems plausible that the interaction of topoisomerase I with the recognition sequence is important in a conserved regulatory process.

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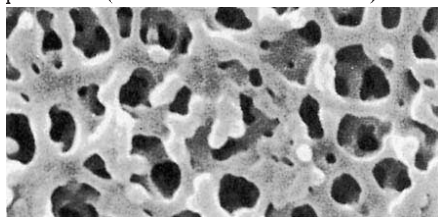
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Spotlight on Israeli science products

As an accompaniment to this week's special feature on Science in Israel, the spotlight falls on a collection of Israeli diagnostics, drugs, research reagents, and medical products.

INTERNATIONAL Diagnostic Laboratories (IDL), located at the centre of the Jerusalem Science-Based Industrial Park, is aiming to attract a portion of the international **lipid diagnostic** market with several new kits (Reader Service No. 100). Earlier this year, IDL entered the US and European markets with immunoturbidimetric kits for the measurement of apolipoproteins A-I and B. As the two principal protein components of HDL and LDL, the presence of these two apolipoproteins serves as an early detection signal for the diagnosis of cardiovascular disease. IDL calibrates its apolipoprotein kit reagents against a CDC international candidate reference serum, and uses high-titre, pooled polyclonal goat anti-human antisera. As a companion to its kits, IDL offers a dedicated bench-top instrument that can read and store calibration curves, and deliver absolute values in mg dl^{-1} for samples prepared any time during the day. Kits for the measurement of apolipoproteins C and E, as well as classical enzyme assays for cholesterol and triglycerides, are currently in the IDL research pipeline.

Gelman Sciences Technology, Inc., in Rehovot, is involved in the production of **medical nonwoven sterile barriers** for use in bandages, gowns, disposable bed sheets, and baby and adult incontinent products (Reader Service No. 101). The



Gelman's micropores prevent the passage of germs while allowing air to circulate freely.

nonwoven barriers have micropores ranging in size from 0.1 to 0.5 μm , and Gelman reports that they retain 99.999 per cent of bacteria while allowing air and water vapour to pass through freely. The nonwovens are prepared by polymerizing small molecules bearing highly reactive acrylate functional groups by exposing them to ultraviolet light. The result is a "breathable" autoclavable sterile barrier that can be mass-produced with relative ease: Gelman reports its pilot plant can turn out the material at 150 feet per minute with a UV light source, and 300 feet per minute using an electron beam apparatus.

Organics Ltd, in Yavne, Israel, is also involved in the development of **health-**

care diagnostics, and has kits for both human and veterinary use (Reader Service No. 102). Organics' line of self-contained ImmunoComb kits consist of a plastic comb and development plate. ImmunoComb kits have been produced for the diagnosis of *Mycoplasma gallisepticum* and *M. synoviae* infections in poultry, and *M. pneumoniae*, Coronaviruses and MHV in mice and rats. Human diagnostics include tests for the detection of IgE, and Rubella and Chlamydia antibodies. Organics is working on a human DNA probe kit for detecting *M. pneumoniae* directly from patient sputum samples, that uses a DNA labelling system involving sulphonation of the DNA followed by recognition by monoclonal antibody detected with a chromogenic reaction with a second enzyme-labelled antibody.

The competition of high titres of immune IgG with IgM for the same antigenic sites can produce false negative results in tests for IgM. Similarly, rheumatoid factor can also produce false results in the presence of IgG. To overcome these problems, Savyon Diagnostics Ltd in Ramat Gan has an **IgG/rf stripping solution** for the treatment of serum before conducting IgM assays (Reader Service No. 103). Savyon's stripping solution is incorporated in its IPAzyme True-IgM series of tests for the detection of IgM specific to *Chlamydia trachomatis* and EEV.

A new brochure, entitled *Immunology Research News*, is now available from International Bio-Technologies Ltd, in Jerusalem (Reader Service No. 104). The free eight-page brochure outlines the uses of IBT's extracellular matrix (ECM) coated labware in **immunological applications**. IBT says the ECM labware is suited for research on the extravasation of neutrophils, T-lymphocytes and macrophages, autoimmune diseases, immune system interactions with malignant cells, and proliferation and differentiation of hematopoietic cells. The brochure also touches on the uses of radioactively labelled ECM coated dishes in matrix degradation studies.

ExtrAvidin is BioMakor's **answer to nonspecific binding problems** (Reader Service No. 105). The Rehovot-based company's ExtrAvidin, developed in conjunction with the Weizmann Institute of Science, combines the high specific activity and sensitivity of avidin with the low

background staining of streptavidin. BioMakor says ExtrAvidin binds biotin with the high affinity of egg white avidin ($K_D = 10^{-15}$) but without the nonspecific binding, such as the staining of mast cells, exhibited by egg white avidin at physiological pH. ExtrAvidin comes unlabelled, or conjugated with alkaline phosphatase, peroxidase, FITC and TRITC.

FRONE is the name of InterPharm Laboratories Ltd's **native beta interferon** product, available as a topical cream or as an intralesional injection (Reader Service No. 106). FRONE has been approved in



InterPharm's beta interferon is approved in some countries for the treatment of herpes.

Argentina, Italy and Israel for the treatment of Herpes virus infections. InterPharm claims that clinical trials have shown that FRONE reduces or eliminates herpes symptoms, prolongs the interval between attacks, and reduces the severity of subsequent attacks. The company, in Ness-Ziona, plans to concentrate next on developing recombinant beta-1 interferon, and on making alpha C and gamma interferon.

Bio-Technology General, with research headquarters in Rehovot, is involved in a range of **biotechnological product** developments (Reader Service No. 107). BTG has a non-methionyl form of human growth hormone that is nearly ready to enter the market. BTG announced last month that its recombinant superoxide dismutase (SOD) has entered Phase II human clinical trials in the US, to ascertain its efficacy in preventing reperfusion damage associated with kidney transplants. SOD has shown promise in animal trials in mopping up the oxygen-free radicals that occur in tissues and the bloodstream when the supply of oxygen is cut off, and that cause tissue damage upon reperfusion. Currently, BTG sells recombinant hyaluronic acid as a moisturizer/lubricant for use in cosmetics, soaps and other personal care products. □

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